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Meeting-Edition

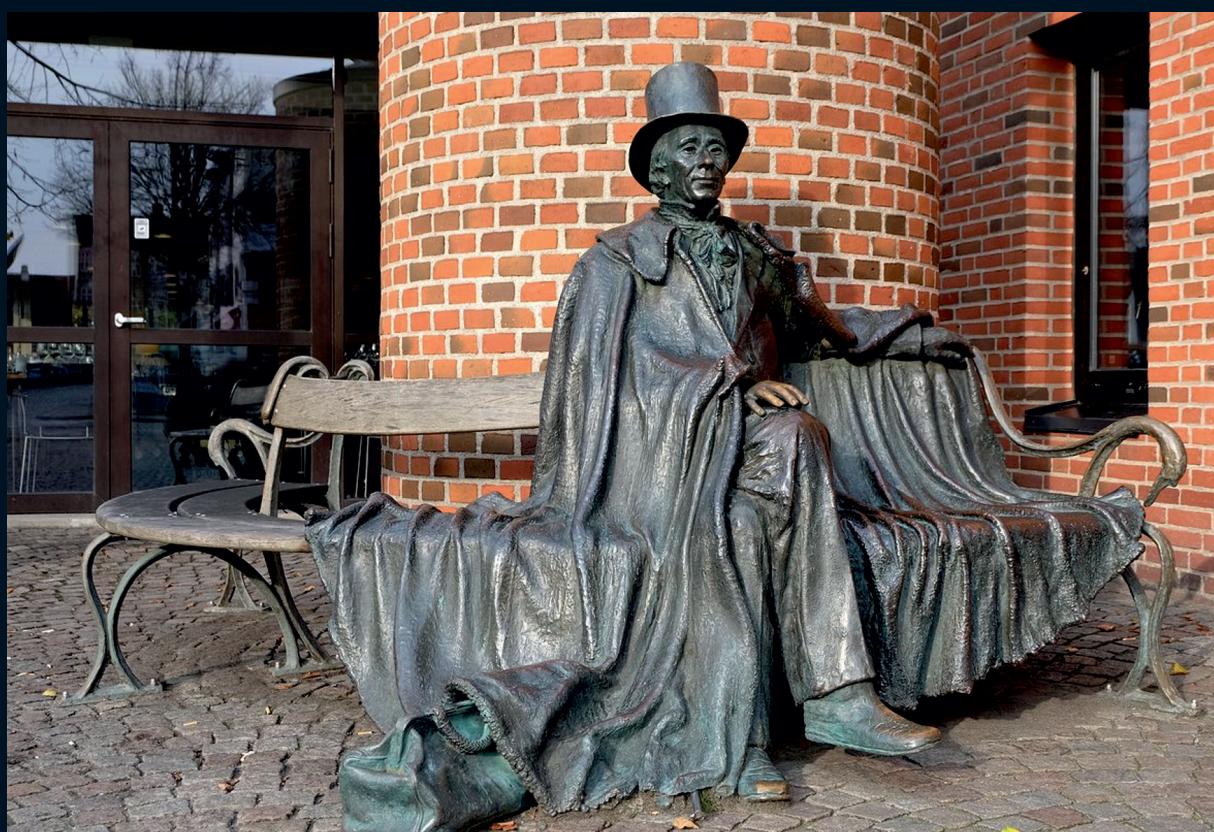
Abstracts

12th European Congress of Neuropathology

Virtual, May 31st — June 3rd, 2021

Organized by the Scandinavian Neuropathological Society (SNS) in conjunction with the European Confederation of Neuropathological Societies (Euro-CNS)

Congress President and Chair Scientific Program Committee:
Bjarne Winther Kristensen, Copenhagen, Denmark



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S1

May
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Euro-CNS
The official Journal of the European Confederation of Neuropathological Societies

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Editorial

Clinical Neuropathology 4-2021

12th European Congress of Neuropathology Welcome from the Congress President

Bjarne Winther Kristensen

Dear Colleagues,

The Organizing and Scientific Program Committees, the Scandinavian Neuropathological Society (SNS) and the European Confederation of Neuropathological Societies (Euro-CNS), are proud to present to you the Abstract Book of the 12th European Congress of Neuropathology. In this issue of Clinical Neuropathology, you will find the regular abstracts as well as the invited speaker abstracts.

Originally, the 12th European Congress of Neuropathology was going to take place in June 2020 in Odense, the warm fairytale city in the heart of Denmark. However, the COVID-19 pandemic led to a complete national lock-down in Denmark less than 3 months before the congress last year.

After postponing the congress to October 2020, the continued COVID-19 pandemic led us to decide to switch to a virtual event – the first virtual European Congress of Neuropathology – to bring neuropathology into this novel virtual age and to share the important knowledge in our field.

Neuropathology is an important discipline. The diseases we cover are critical for our patients and their relatives and our focus on novel diagnostic approaches including molecular mechanisms explaining pathological and clinical manifestations of neurological diseases is highly needed.

Together with our Scientific Program Committee with both Scandinavians and experts from all over Europe and representing all the different areas of expertise in neuropathology, we have the pleasure to present a very diverse program.

While our program is research driven with 6 plenary lectures and 13 symposia intended to inform the delegates about the most recent developments, research, and results, the 9 workshops will illustrate the more practical approach of how new knowledge is integrated into daily neuropathology. Importantly and being of high relevance, we have a symposium on the neuropathological aspects of COVID-19.

The purpose of the Free Communications and Poster Quick Pitches (short oral abstract presentations) is to integrate more deeply into the congress all the important knowledge of the 245 abstracts submitted for the congress. The ePoster platform, the Learning Toolbox, connects the delegates with the poster presenters in a unique way. Questions can be asked realtime via chat - so poster presenters will be easy to reach and leave the meeting with new connections, knowing that many have seen their presentations.

I deeply appreciate all the help and support from the Organizing and Scientific Committees, from Euro-CNS and SNS and many other sides to organize the 12th European Congress of neuropathology. We are

ISSN 0722-5091

DOI 10.5414/NPP40215
e-pub: May 20, 2021

extremely grateful for support from both our scientific and industry sponsors.

We trust that this valuable compilation of speaker and regular abstracts is useful for you. Note that the ePoster platform is open for all registered delegates and that you can see the posters and communicate with the authors by chat.

We hope to welcome you to the 12th European Congress of Neuropathology, on a road leading to new collaborations and future discoveries.

Bjarne Winther Kristensen
Congress President
Chairman of the Organizing and Scientific Committees
President, Scandinavian Neuropathological Society (SNS)
Vice President of the European Confederation of Neuropathological Societies (Euro-CNS)

Practical information concerning the Congress and ePosters (poster presentations of regular abstracts)

Congress website with information about the program, registration and exhibits:
www.ecnp2021.dk

The event website for attending the virtual congress as of May 31:
https://crowdcomms.com/ecnp12/

ePosters are accessible from within the event website for registered participants

Our abstract authors are looking forward to hearing from you!

Most content of the congress will be recorded and made available on-demand within 24 hours of the sessions. The content will remain online until 3 months after the event.

Clinical Neuropathology, Vol. 40 – No. 4/2021 (217-241)

clinical neuropathology

Abstracts

ISSN 0722-5091

DOI 10.5414/NPP40217
epub: May 20, 2021

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! 12th European Congress of Neuropathology: The regular Abstracts are available in the Online Supplementary issue 1/2021: clinneuropathol.com or by DOI 10.5414/NPP40S01 !

Speaker Abstracts

Plenary Lectures

P1

Brain tumor diagnosis in transition – from single parameter analyses to multi-omics

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Diagnosis of human brain tumors relies on morphological evaluation, immunohistochemistry and molecular tests targeting tumor specific alterations. Molecular testing most heavily relies on variations of FISH and DNA sequencing approaches. In the last decade molecular platforms addressing parameters on a global range have been developed. These technologies now start entering diagnostic standard procedures. Already established is next generation sequencing (NGS) of DNA, mostly as NGS panels addressing diagnostically or therapeutically relevant genes in numbers ranging from 20 to several hundred. More recently, the discovery of an increasing number of gene fusions also in human brain tumors renders RNA-sequencing a relevant platform. Promoter DNA meth-

ylation status has emerged as highly valuable for categorizing tumors and is used for tumor classification at an increasing number of diagnostic centers. Due to a high intratumoral and longitudinal stability of promoter methylation patterns, this platform may develop as a standard for classification. Currently analysis of promoter DNA methylation status is a highly prolific tool for identifying novel tumor entities. And last, proteomic analysis is on the diagnostic horizon. This approach will allow a highly refined landscape of tumor specific protein prevalence and may as such serve as a source for developing simple immunohistochemical tests for brain tumor discrimination. Further, the potential of identifying and quantifying key regulatory phosphoproteins may also have major impact on postoperative treatment. Current role of omics platforms and their future potential in brain tumor diagnosis will be discussed.

Keywords: Brain tumor diagnosis – multi-omics

P2

Lessons learned from brain banking for neurodegenerative diseases

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The brain bank for neurodegenerative disorders at Mayo Clinic in Jacksonville, Florida has grown to be one of the largest brain banks for neurodegenerative disorders in the world, where almost all of the cases have been evaluated by a single individual. The brain bank provides a valuable resource of samples to be shared with international and multicenter genomic and transcriptomic studies. A guiding principle in has been quantitative approaches, including counts of senile plaques, neurofibrillary tangles (NFT) and Lewy bodies, and semi-quantitative scores of neuronal and glial lesions in tauopathies (tangles/pretangles, tufted astrocytes, astrocytic plaques, coiled bodies). Analysis of NFT counts with a mathematical algorithm in a large number of pathologically-confirmed cases identified three distinct subtypes of AD – hippocampal sparing, limbic predominant and typical. Heterogeneity of AD has recently been confirmed in a longitudinal tau PET imaging study using unbiased spatiotemporal cluster analyses. Semiquantitative data has led to recognition of subtypes of PSP with distinct clinical presentations and genetic associations, such as the LRRK2 locus. Systematic analyses lay the groundwork for future discoveries. Neuronal loss in the hippocampus (HpScl) that occurs in the absence of hypoxic-ischemic injury was reported in 1991 in a series of prospectively studied patients. Discovery of TDP-43 led to an explanation for HpScl in the elderly. Genetic evidence (GRN and TMEM106B) suggests that HpScl is a form of old-age FTLD-TDP.

Keywords: brain bank – quantitative – neurodegenerative

P3

Brain somatic mutations in focal cortical dysplasias

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Introduction: Focal cortical malformations are important causes of severe pediatric drug-resistant epilepsies subjected to neurosurgery. Molecular studies have revealed somatic mosaicism in a spectrum of focal cortical malformations, such as focal cortical dysplasia (FCD) and mild malformation of cortical development (mMCD). Objectives: To elucidate the genetic cause of FCD/HME. Methods: We performed ultra-deep targeted gene sequencing on matched blood-brain resected samples to search for low-allele frequency variants in a panel of mTOR pathway and FCD genes. We next searched for specific mutations by digital-droplet PCR (ddPCR) in the CSF from 12 patients. Results: The molecular signature of FCD2 is the presence of mosaic variants in a set of genes belonging to the mTOR signaling cascade. Two types of mutational events may explain the occurrence of a dysplasia: single activating variants in MTOR or in genes encoding upstream activators of the pathway (AKT3, PIK3CA, RHEB), or "double-hit" inactivating variants (SNV or loss-of-heterozygosity) in repressors of the pathway (DEPDC5, NPRL2/3, TSC1/2). Analysis of microdissected cells demonstrated that only DNs and BCs in FCD2 carry the pathogenic variants. Using targeted digital droplet PCR, we were able to detect the presence of somatic variants in CSF-derived circulating cell-free DNA samples in 3/12 epileptic patients. Our findings suggest potential opportunities for clinical use of CSF to establish a genetic diagnosis even when brain tissue is not available. We also report that 30% of mMCD patients and a large fraction of MOGHE cases carried somatic loss-of-function SLC35A2-variants involving the glycosylation pathway. Conclusion: Our novel findings represent an important advancement in understanding the genetic etiology and mechanisms underlying epilepsies associated with focal cortical dysplasia.

Keywords: Epilepsy – cortical malformation – focal cortical dysplasia – somatic mutations – mTOR pathway

P4

The invasive behavior of glioma cells in the CNS and their biological characteristics

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Extensive tumor cell invasion into the brain parenchyma is a primary characteristic of gliomas. Tumor recurrence after surgery and chemo/radiotherapy, can to a large extent

be attributed to the remaining invasive tumor mass. Although extensive invasion takes place in the brain, gliomas rarely metastasize out of the CNS. This is in contrast to secondary brain tumors that metastasize to the brain, – but once within the CNS, limited invasion takes place. In order to get a comprehensive molecular and dynamic picture of brain tumor invasion in real-time, several ex-vivo invasion models have been developed including invasion into brain organoids (BOs) derived from fetal rat brain tissue and from human pluripotent stem cells. We have performed comprehensive molecular analyses (transcriptomics, proteomics and metabolomics) on the BO systems focusing on their developmental path to potential maturity. We show that fetal rat brain BOs develop into terminally differentiated mature CNS structures whereas BOs developed from IPSc do not reach a terminal differentiation stage. In order to delineate the invasive process, we confronted our mature BOs with a number of highly characterized patient derived glioma organoids where tumor invasion parameters (migratory paths, speed of invasion, cellular heterogeneity within the invasive compartment) where assessed in detail. We also show that organoids derived from different GBM patients have different modes of invasion which were further validated in ortotopic xenograft models in vivo. Finally, we show that this ex-vivo avatar invasion system can be exploited to assess therapeutic interventions towards the invasive compartment.

Keywords: Glioma – invasion – metastasis

P5

Polyglucosan storage in muscle and brain – disease entities and pathogenesis

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As evidenced by patients with glycogenosis type 0, glycogen is dispensable for skeletal muscle and brain function as long as provision of glucose by the liver is maintained. On the other hand, accumulation of insoluble and indigestible glycogen (polyglucosan) in muscle or brain is pathogenic. Polyglucosans are glycogen molecules with overlong branches, which, as in starch, wind around each other and drive affected molecules to precipitate and over time accumulate. These accumulations are associated with inflammation and degeneration resulting in progressive skeletal muscle and/or nervous system dysfunction. The prototypical and easiest to understand polyglucosanosis is glycogen branching Enzyme deficiency. Next would be increased activity of the glycogen chain elongating enzyme glycogen synthase by gain-of-function mutations. Glycogen synthase activity can also rise indirectly either through loss of its inhibitory kinase AMPK, or increase in its allosteric activator glucose 6-phosphate following blockages of glycolysis such as in phosphofructokinase deficiency. Polyglucosans also form in the absence of the glycogen synthesis primer glycogenin. Polyglucosans arise in the absence of the glycogen phosphatase laforin or the latter's interacting and disease-critical ubiquitin E3 ligase partner malin. Polyglucosans form following failure of the linear ubiquitin chain assembly complex such as in deficiency of the RBCK1 ubiquitin E3 ligase. Finally, deficiency of yet another ubiquitin E3 ligase, KLHL24, also causes polyglucosan generation. From the above it is clear that ubiquitination dependent mechanisms abound in the control of glycogen architecture. In this presentation, I will attempt to convey the latest information on the latter, following an overview of all the known polyglucosan storage diseases.

Keywords: storage diseases – polyglucosans – ubiquitination

P6

Deciphering phenotypic variability and transmission properties of human prion diseases

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Prion diseases are rare neurodegenerative disorders characterized by tissue deposition of heterogeneous aggregates of abnormally folded protease-resistant prion protein (PrPSc), extensive phenotypic heterogeneity, and variable efficiency of disease propagation. As in classical infectious disorders, experimental transmissions led to the isolation of prion strains with distinct biological properties, including specific regional targeting and pattern of intracerebral spreading. Numerous evidence indicated that different PrPSc conformers encipher the phenotypic variants related to prion strains, a concept now applied to all protein amyloids showing polymorphic structures and evidence of propagation by seeding. The characterization of distinct isoforms of PrPSc that strongly correlate with the disease phenotype led to the concept of molecular strain typing, in which different PrPSc "types" represent strain-specific markers. PrPSc forms in Creutzfeldt-Jakob disease (CJD) and fatal insomnia mainly comprise glycosylated and GPI-anchored C-terminal fragments of 21 – 19 kDa named type 1 and type 2. The two PrPSc isoforms in combination with the genotype (methionine, M or valine, V) at the polymorphic codon 129 in the prion protein gene largely identify the six major clinicopathological subtypes (e.g., MM1, MM2, VV1, etc.) included in current sporadic CJD classification. In contrast, PrPSc aggregates in Gerstmann-Sträussler-Scheinker disease and variably protease-sensitive prionopathy mainly comprise C-terminally truncated, unglycosylated and anchorless fragments of 7 – 11 kDa, which result in a slower disease progression, increased formation of

amyloid plaques, and reduced transmissibility. Besides the phenotypic characterization of the most common variants, the description of atypical and mixed phenotypes with co-occurrence of PrPSc types, the comparison of CJD cases with different etiology (sporadic vs. iatrogenic vs. genetic), and the results of experimental transmission studies, including the correlation between PrPSc properties and transmission rate in the most common forms have more recently provided further insight into our knowledge of the phenotypic spectrum of human prion disease and its molecular basis.

Keywords: prion disease – CJD – prion strains – PrPSc – classification

Symposia

Symposium 1 Cerebrovascular diseases

S1.1

Post-stroke inflammation – target or tool for therapy

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Inflammation is currently considered a prime target for the development of new stroke therapies. In the acute phase of ischemic stroke, microglia are activated, and then circulating immune cells invade the peri-infarct and infarct core. Resident and infiltrating cells together orchestrate the post-stroke inflammatory response, communicating with each other and the ischemic neurons, through soluble and membrane-bound signaling molecules, including cytokines. Inflammation can be both detrimental and beneficial at particular stages after a stroke. While it can contribute to the expansion of the infarct, it is also responsible for infarct resolution and influences remodeling and repair. Several pre-clinical and clinical proof-of-concept studies have suggested the effectiveness of pharmacological interventions that target inflammation post-stroke. Experimental evidence shows that targeting certain inflammatory cytokines, such as tumor necrosis factor, interleukin (IL)-1, and IL-6 holds promise. However, as these cytokines possess non-redundant protective and immunoregulatory functions, their neutralization or augmentation carries a risk of unwanted side effects, and clinical translation is, therefore, challenging. This talk focuses on the cell biology of the post-stroke inflammatory response and presents pre-clinical pharmacological interventions targeting inflammation in the acute phase after a stroke that may be used alone or in combination with recanalization therapies.

Keywords: Stroke – cytokines – microglia

S1.2

White matter in familial small vessel diseases: CADASIL

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Introduction: CADASIL is a genetic paradigm of small vessel disease of the brain caused by mutations in NOTCH3 that stereotypically lead to the accumulation of NOTCH3 around smooth muscle cells and pericytes of small blood vessels. One of the earliest and most consistent features of CADASIL observed is the presence of white matter (WM) lesions. Objective: To characterize the pathological substrate of WM lesions at the early stage of the disease. Methods: We used TgNotch3R169C mice, a well-established model of CADASIL that recapitulates the early stage of the disease. We analyzed post-mortem brain tissue from CADASIL and control mice. We injected tracers to assess the blood-brain barrier integrity. Results: CADASIL mice exhibited WM changes initially (6 months) in the form of increased punctate staining using a specific monoclonal antibody (SMI-94) against myelin basic protein – which is thought to mark uncompacted or degraded myelin – and later in life (20 months) as a pallor and vacuolization of WM tracts. Further analysis using high pressure freezing and freeze substitution in combination with high-resolution electron microscopy revealed significant splitting of myelin layers and enlargement of the inner tongue of small caliber axons from the age of 6 months, then vesiculation of the inner tongue and myelin sheath thinning at 15 months of age. Remarkably, there was no reduction in the number of mature oligodendrocytes and the number of activated microglial cells was unchanged at any age. Moreover, there was no extravasation of endogenous fibrinogen, nor of injected small or large tracers in the white matter, and both the pericyte number and coverage were unchanged. Conclusion: We conclude that early WM lesions in CADASIL affect first and foremost the myelin sheath and the inner tongue, suggestive of a primary myelin injury. We propose that those defects are consistent with a hypoxia/ischemia mechanism.

Keywords: CADASIL – white matter – myelin

S1.3

White matter disease – small vessel pathology and more

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The term cerebral white matter disease (WMD) connotes a progressive deterioration of the white matter, irrespective of cause. Many etiologies are claimed, none however is singled out as the leading cause of WMD. A cerebrovascular cause and a small vessel character of disease would be the first mentioned category of WMD. Accumulated clinical and radiological data convene with the neuropathologic knowledge to proclaim WMD as predominantly a manifestation of cerebrovascular disease. This, in turn, shows to hold a

spectrum of vascular disorders, from known widespread diseases to rare conditions associated with specific mutations. Other types of WMD are however also prevalent and among these, the proteinopathies appear. In both tauopathies and synucleinopathies, WMD may be apparent, even markedly severe, and sometimes bearing the brunt of degeneration in the brain, without vascular pathology, at least not obvious. The frontotemporal lobar degeneration group of diseases present a variety of WMD being specifically accentuated along certain white matter tracts. In Alzheimer's disease, an apparent cortical degeneration accentuated in the temporo-parietal regions is most often accompanied by a mild to severe subjacent white matter pathology, primarily a demyelination being secondary to neuronal degeneration. The frontal lobes, most often being less severely affected by the cortical neurodegeneration, will later undergo a WMD. This WMD does not match the overlying cortical disease, but more to the vascular pathology: the more amyloid angiopathy in the meningo-cortical vessels, the more the white matter damage. The brunt of damage from this secondary process affects the frontal lobe white matter and is of vascular-ischemic type. Here thus, the neurodegenerative and vascular-ischemic diseases convene.

Keywords: WMD – vascular – myelin

Symposium 2 Genetics of neurodegeneration

S2.1

Clues to the etiology of neurodegenerative diseases from genomic analysis

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In this age of genome wide associations, we now know many causative and many risk genes for all the major neurodegenerative diseases. We are only now, however, beginning to make sense of all this new information. In my talk I will attempt to bring together current knowledge from both mendelian and risk genes to produce a consistent outline of pathogenetic mechanisms. I will start by reviewing the literature from our group and others on genetic risk and I will argue that in all cases, mendelian disease can be caused by overproduction of proteins (APP in AD, synuclein in PD and tau in the tauopathies) and that much of the risk in "sporadic disease is predisposed by failures to clear these proteins: Abeta by microglial failure, synuclein by lysosome failure and tau by ubiquitin failure. I will suggest that these findings suggest that the important common feature of these diseases is an age-related decline in homeostatic mechanisms and I will discuss treatment implications.

Keywords: Alzheimer's Disease – Parkinson's Disease – frontotemporal dementia

S2.2

Pathology of genetic and sporadic Parkinson's disease

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The classical pathological features of sporadic Parkinson's disease (PD), detectable with a hematoxylin and eosin (H&E) stain, are neuronal loss, gliosis and the presence of intraneuronal inclusions, known as Lewy bodies (LB). LB can be found in a number of different subcortical and brainstem nuclei, most notably the substantia nigra, and also in the cortex. The vast majority of PD cases are sporadic but there are also some inherited forms of the disease, the first of which was discovered, in the late 1990s, to be related to changes in the SNCA gene. α-synuclein, the gene product, was subsequently found to be present in LB and Lewy neurites. α-synuclein immunostaining is now the standard method for detection of LB-associated pathology and reveals a much wider distribution of pathology in the PD brain than can be seen with an H&E. α-synuclein pathology spreads through the brain in a fairly stereotypical manner which maps well with the emergence of non-motor symptoms. The degree of spread is assessed using the Braak staging system from I-VI with the earlier stages being in the brainstem and the later stages in the cortex. Based on these observations, PD has been classed as an α-synucleinopathy. However, more than 20 different PARK genes have now been identified and, where autopsy data is available, it is clear that the pathology in these cases can be very variable and does not always include LB. This raises some interesting questions about the primacy of LB in the pathogenesis of clinical PD.

Keywords: Parkinson's – α-synuclein – pathology – genetics

S2.3

Genetics of MND/ALS – from genes to translational approaches

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Motor neuron disease (ALS/MND) is one of the 3 commonest adult-onset neurodegenerative disorders with a life-time risk of approximately 1 in 300. The clinical features are caused by injury and cell death usually of both upper motor neurons in the brain and lower motor neuron groups in the brainstem and spinal cord. Death in most patients results from neuromuscular respiratory failure. The heterogeneity and complexity of MND has posed a challenge for neuroprotective therapy development. This lecture will cover 3 areas of topical interest in relation to ALS/MND. 1) Genetics of ALS/MND: ALS/MND is a genetically heterogeneous condition. At least 30 genes are known to contribute to the

development of familial ALS/MND. Advances in genetics are now allowing us to subclassify the condition better, allowing prospects for a more personalized medicine approach. It is also now apparent that genetic factors also contribute to apparently sporadic disease with a rare variant architecture, as demonstrated when routine screening for known genes is undertaken. The presence of more than one pathogenic mutation in an individual promotes a more aggressive disease course, with earlier age of onset. 2) ALS/MND caused by mutations in the SOD1 gene has generated the deepest understanding of disease biology to date. However, the reasons why the development of neuroprotective therapies for MND has been slow include over-reliance on the SOD1 transgenic mouse model which represents only 2% of MND cases. Although protein aggregation is a feature of SOD1 ALS/MND, this subtype is not a TDP-43 proteinopathy which is a pathological hallmark in the majority of cases. 3) The next section will review the positive translational approaches emerging from genetic therapy trials now in progress for MND patients with SOD1 and C9orf72 gene mutations. An exciting factor emerging from the SOD1 anti-sense oligonucleotide trial are the long-awaited biomarkers of therapeutic efficacy, including SOD1 protein levels in CSF and neurofilament levels in CSF and plasma.

Keywords: ALS/MND – genetics – genetic therapy trials

Symposium 3 CNS tumor precision oncology – what is important in the neuropathological setting?

S3.1

The laboratory setup needed for CNS tumor precision oncology

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The requirements on molecular work-up of diagnostic specimen are increasing. With the new WHO classification, several novel markers, including novel types as gene fusions, become indispensable to render clear diagnoses. The talk will review the spectrum of markers that need to be assessed, with the respective technologies currently available and in development. Besides technical aspects, also other practical considerations as throughout and efficient use of resources will be discussed.

Keywords: molecular neuropathology – gene fusions – panel sequencing – methylation – WHO classification

S3.2

Experience and results from a precision oncology phase I unit

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The development of targeted therapies against oncogenic activated molecular targets have changed clinical cancer care. Druggable oncogenic drivers have been reported in specific tumor types such as lung cancer, melanoma, colorectal cancer, and gastrointestinal stromal tumor. Specific drivers have also been identified across different tumor, and the first tumor agnostic drugs have recently been approved. Next-generation sequencing (NGS) allows rapid detection of clinically actionable aberrations. Increasing availability and decreasing cost of NGS have increased pace of the development within individualized cancer treatment guided by genomic profiling. The major goal of precision medicine is to identify the mechanisms of cancer progression at the individual level to apply targeted treatment. Traditionally, patients with exhausted treatment options have been candidates for early clinical trials where the aims have been to establish dose and assess toxicity in unselected patients. Today, genomic profiling to select patients in the early trial setting have improved the outcome for such patients and speeded up drug development. Major Phase 1 Units in Europe and USA have implemented screening programs with extensive molecular profiling to allocate the right patient to the right trial. Most recently, NGS is being implemented earlier in the treatment of patients, also in GBM, in order to identify patients that may benefit from emerging targeted agents. Among relevant oncogenic drivers are BRAF-V600E, IHD1, FGFR-fusions and NTRK-fusions. The setup and experiences from the Phase 1 Unit in Copenhagen will be presented.

Keywords: NGS – drug development – GBM

S3.3

Molecular diagnostics of brain tumors – current practice and the next frontiers

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The discovery of molecular biomarkers in brain tumors has made significant progress over the last 10 years. Key discoveries were IDH,

BRAF, histone mutations, which now define tumor entities or unify pathogenetically similar CNS neoplasms. The development of a methylome-based tumor classification system has generated further insight into the cell of origin and pathogenesis of many tumors which were difficult, or impossible to characterise and classify previously. In addition to tumor classification (i.e., more precise diagnosis), methylation profiling also serves prognostication, for example in meningiomas. Technological developments such as next-generation sequencing has added further accuracy and depth of brain tumor diagnostics, and prognostication. All these developments present continuous challenges to the diagnostic neuropathologist, to the pathology laboratory, and in the wider context to the healthcare systems, to keep up with the technological advancements, and to provide funding for adequate infrastructure, with equal access to all patients. I will present our approach to molecular diagnostics of brain tumors, discussing how we balance clinical needs, diagnostic precision, and cost-effectiveness. Finally, there will be a brief discussion of the Nanopore technology which is in an early phase of implementation into the clinical workflow, to provide a new approach to rapid and precise brain tumor diagnostics.

Keywords: Brain tumor classification – precision diagnosis – IDH – BRAF – methylation profiling

Symposium 4 The 2021 WHO CNS tumor classification: The Fifth!

S4.1

From cIMPACT-NOW to WHO 2021 classification of CNS tumors

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In the WHO 2016 classification of CNS tumors, molecular characteristics were for the first time formally incorporated into the definition of some of these neoplasms. Acknowledging that further elucidation of the biology of CNS tumors would continue to occur at a rapid pace, late in 2016 a group of neuropathology and neuro-oncology experts established the consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW). A major goal of this consortium was to communicate discoveries with important implications for clinical practice and for the design and interpretation of clinical trials in advance of the release of a new WHO CNS tumor classification. Over the past four years, seven cIMPACT-NOW updates have been published in neuropathology journals with recommendations for improved diagnosis and classification of CNS tumors. Salient recommendations include those providing guidance for how (even in the absence of highest malignancy grade histologically), molecular markers can be used to reach a diagnosis of glioblastoma, IDH–wildtype, or astrocytoma, IDH-mutant, grade 4. Other cIMPACT-NOW updates proposed several changes regarding diagnostic principles and nomenclature, or suggested tumor types that emerged as mature enough to deserve a separate status in a next WHO classification. While most of these cIMPACT-NOW recommendations are indeed incorporated in the WHO 2021 CNS tumor classification, because of even more recent insights some modifications of these recommendations are introduced in the WHO classification as well. It is foreseen that, also after the publication of the WHO 2021 classification, the efforts of cIMPACT-NOW will continue to be very helpful for optimal (incl. evidence-based, balanced, and rapid) translation of novel insights into clinical diagnostics, and thereby for providing the best possible care to patients suffering from a CNS tumor.

Keywords: WHO classification – CNS tumor – cIMPACT-NOW – molecular diagnostics

S4.2

The WHO 2021 classification of diffuse gliomas (adult & pediatric)

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This educational lecture will provide an update on the classification of diffuse gliomas according to the recent recommendations of the cIMPACT-NOW consortium and the upcoming 5th edition of the World Health Organization (WHO) classification of central nervous system tumors 2021. Major changes in comparison to the WHO classification 2016 will be illustrated and novel tumor types of diffuse gliomas will be introduced. Diffuse gliomas in adult patients, i.e. "adult-type diffuse gliomas", basically include three major tumor types, namely Astrocytoma, IDH-mutant, oligodendroglioma, IDH-mutant and 1p/19q-codeleted, and glioblastoma, IDH-wildtype. IDH-mutant astrocytomas are graded into WHO grades 2, 3 or 4 depending on histological features of malignancy. In addition, homozygous deletion of the CDKN2A/B gene locus is considered as an independent molecular indicator of WHO grade 4 in this tumor type. The previous terms "anaplastic astrocytoma, IDH-mutant" and "glioblastoma, IDH-mutant" are no longer recommended. IDH-mutant and 1p/19q-codeleted oligodendrogliomas are stratified into WHO grade 2 or 3 depending on the presence or absence of histological features of anaplasia. The diagnostic criteria for glioblastoma, IDH-wildtype have been broadened by including TERT promoter mutation, EGFR amplification and/or gain of chromosome 7 combined with loss of chromosome 10 (+7/–10) as diagnostic markers indicating WHO grade 4 in an IDH-wildtype diffuse astrocytoma even in the absence of

microvascular proliferation and necrosis. Pediatric-type diffuse gliomas are divided into eight distinct tumor types, with 4 types each considered as high-grade or low-grade pediatric-type diffuse gliomas. The high-grade group includes Diffuse hemispheric glioma, H3.3 G34-mutant, Diffuse midline glioma, H3-K27-altered, High-grade pediatric-type diffuse glioma, IDH-wildtype and H3-wildtype (all considered as WHO grade 4), and Infant-type hemispheric glioma, a rare tumor in infants that is characterized by gene fusions involving the NTRK1-3, ROS1, ALK or MET genes. Pediatric-type low-grade diffuse gliomas include Diffuse astrocytoma, MYB or MYBL1-altered, Angiocentric glioma, Pleomorphic low-grade neuroepithelial tumor of the young (PLNTY), and Diffuse glioma, MAPK pathway-altered. The essential and desirable histological and molecular diagnostic criteria for each of these distinct tumor types will be presented as defined in the WHO classification 2021. The importance of novel molecular diagnostic approaches, in particular DNA methylome and next generation sequencing analyses, will also be addressed.

Keywords: diffuse gliomas – integrated diagnostics – WHO classification 2021

S4.3

The WHO 2021 classification of circumscribed astrocytic gliomas and glioneuronal tumors

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Background: Since the WHO 2016 edition, major advances have been done regarding genetic characterization of circumscribed astrocytic gliomas and glioneuronal tumors and in 2020 a summary of new entities recognized by the cIMPACT-NOW consortium that precludes the WHO 2021 classification has been published. Results: The WHO 2021 introduces a very large category of tumors entitled "Gliomas, Glioneuronal and Neuronal tumors". In this large category, the subgroup "circumscribed astrocytic gliomas" encompasses 6 entities, two of them being new: the "high-grade astrocytoma with piloid features" and the "Astroblastoma, MN1-altered", whereas "chordoid glioma of the third ventricle" has been renamed as "chordoid glioma". The other entities remain unchanged. A new entity has been recognized among glioneuronal tumors: "Myxoid glioneuronal tumor" whereas "Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters" is considered as a new provisional entity. Besides, two subtypes with distinct DNA-methylation profile are described for diffuse leptomeningeal glioneuronal tumors: DLGNT-MC-1 and DLGNT-MC-2. All these new entities and subtypes display characteristic clinical, pathological and molecular features and also a specific DNA-methylation profile. Conclusions: The WHO 2021 classification has provided major advances in the field of circumscribed astrocytic gliomas and glioneuronal tumors and incorporates molecular approaches for the diagnosis of these tumors in order to provide an integrated (and layered) histomolecular diagnosis. It also recognizes DNA-methylation profiling as a powerful diagnostic tool that may also be used as a surrogate marker for some genetic events. Essential and desirable diagnostic criteria are provided for each tumor type.

Keywords: Circumscribed astrocytic gliomas and glioneuronal tumors – WHO 2021 – integrated histomolecular diagnosis

Symposium 5 Mechanisms of brain Inflammation

S5.1

Pathways of drainage of interstitial and cerebrospinal fluids from the brain: significance for neurological diseases

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Introduction: Cerebrospinal fluid (CSF) is eliminated across arachnoid villi into venous blood and there is lymphatic drainage along cranial nerves or dural lymphatics. In the absence of conventional lymphatics, interstitial fluid (ISF) from the brain parenchyma drains along basement membranes in walls of capillaries and arteries as Intramural Peri-Arterial Drainage (IPAD) to cervical lymph nodes. Objectives/purpose: To 1) Determine if IPAD fails with age or possession of Apolipoprotein E4 (APOE4) or after immunization against amyloid-β (Aβ); 2) Analyze the anatomical pathways for the relation between CSF and ISF. Methods: 1) stereotaxic intracerebral injections of fluorescent soluble dextrans or Aβ were performed in mice that were old, or APOE4 positive or immunized against a protein inoculated into the brain; the distribution of the tracers was analyzed by confocal or transmission electron microscopy. 2) fluorescent soluble Aβ was injected into cisternal CSF and its distribution in the brain parenchyma analyzed by confocal microscopy. Results: IPAD fails with increasing age, possession of APOE4 genotype and after immunization against a cerebral protein. Within 5 minutes of their injection into the CSF, tracers enter the brain parenchyma along pial-glial basement membranes on the outer aspects of cortical arteries and within 30 min are observed in IPAD pathways. Conclusion: Failure of IPAD

is a key factor in the pathogenesis of cerebral amyloid angiopathy (CAA) and Alzheimer's disease. Convective influx of CSF into brain parenchyma can be harnessed as a strategy for delivery of therapeutics to cerebral arterial walls.

Keywords: arterial basement membranes – drainage of interstitial and cerebrospinal fluid

S5.2

Immune cell interaction with the blood-brain barrier in the pathogenesis of inflammation

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Background: Blood-brain barrier (BBB) breakdown and immune cell infiltration into the central nervous system (CNS) are amongst the earliest pathological hallmarks observed in multiple sclerosis (MS). It is generally thought that BBB breakdown is a consequence of neuroinflammation. Objective: We challenge this view. Results and methods: We show that the BBB plays an active role in directing T cell migration to paracellular or transcellular sites of diapedesis depending on its inflammatory status. Combining in vitro live cell imaging of T-cell migration across primary mouse brain microvascular endothelial cells under physiological flow with serial block face scanning electron microscopy (SBF-SEM) we have recently identified BBB tricellular junctions as novel sites for T-cell diapedesis across the BBB. Making use of human induced pluripotent stem cell (hiPSCs) derived from healthy controls or MS patients and differentiating those to brain microvascular endothelial (BMEC)-like cells as in vitro model of the BBB we found that the MS-derived BMEC-like cells showed impaired junctional integrity and barrier properties and displayed an inflammatory phenotype with increased adhesion molecule expression and immune cell interactions when compared to HC-derived BMEC-like cells. Conclusion: Taken together our findings underscore the active role of the BBB in neuroinflammation and suggest that intrinsic BBB dysfunction may contribute to MS pathogenesis.

Keywords: blood-brain barrier – tricellular junctions – hiPSC-derived BMEC-like cells

S5.3

Pathology of different human inflammatory CNS diseases – an expanding spectrum

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Traditionally inflammatory diseases of the central nervous system have been grouped into infectious diseases and putative autoimmune diseases. For therapeutic reasons the identification of an infectious agent, which drives inflammation and damage in the brain and spinal cord, is important, but in addition knowledge about immunological mechanisms allowed to define new diseases and to ameliorate immune mediated tissue damage. Immune surveillance of the brain involves CD4+ T-cells and CD8+ T-lymphocytes with the help of B-lymphocytes. After the acute inflammatory episode, they remain in the nervous tissue for prolonged time as tissue resident effector memory cells and provide an effective barrier for re-infection. However, these cells also may drive chronic autoimmune disease. This mechanism plays a major role in virus infections of the nervous system as well as in many autoimmune diseases, such as multiple sclerosis or paraneoplastic diseases. Another type of inflammation is mediated by antibodies, expressed on the surface of CNS antigens, which in cooperation with T-cell mediated inflammation, contribute to tissue injury by activation of complement or their interaction with activated macrophages. These antibodies are also instrumental in the defense against brain infections and induce disease-specific tissue injury in various autoimmune diseases, including neuromyelitis optica spectrum disorders. Auto-antibodies can in addition directly trigger brain disease by interfering with neurotransmitter receptors or ion channels or may just serve as diagnostic disease markers. Finally, mechanisms of innate immunity propagate disease and brain damage in bacterial meningitis and are instrumental in the induction of bystander damage in infectious and autoimmune diseases.

Keywords: autoimmunity – infection – inflammation – lymphocytes – antibodies

Symposium 6 Immuno-oncology

S6.1

The inflammatory microenvironment as a therapeutic target in glioma

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The inflammatory microenvironment of glioma is characterized by a diverse composition of immunological cells. T cells are in comparison rarely observed while microglia cells/macrophages are frequent. Several immune suppressive factors are initiated by glioma cells, resulting in an overall immune suppressive microenvironment. A particular challenge in the successful utilization of immune modulatory mechanisms for glioma treatment, is the location in the brain and the resulting strong immune regulation. Antigen transport and presentation as well as T cell influx are potentially restricted. On the other hand, microglia/macrophages in the inflammatory tumor microenvironment have tumor suppressing as well as tumor promoting functions. Several immune modulatory therapeutic mechanisms have been explored; however, a clinically meaningful impact could not be observed for any single mechanism applica-

tions. Indeed, a combined approach, targeting several steps of the cancer -immune cycle might be clinically more promising. Further, the role of systemic immune suppression needs to be addressed to establish successful immune modulatory treatments in glioma.

Keywords: Tumor infiltrating T cells – microglia; macrophages – immune checkpoint inhibitor

S6.2

Genetic changes and T-cell infiltration in gliomas

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Activation of the host's immune system has emerged as an exciting new treatment option for cancer patients. Unfortunately, such immune therapies have thus-far not provided clinical benefit for glioma patients. We have recently shown that CD3 and CD8 T-cell numbers are significantly fewer in numbers in lower grade gliomas (LGGs, oligodendrogliomas and astrocytomas) and, in contrast to glioblastomas, show reduced extravasation. In lower grade gliomas, we show that T-cells remain trapped within the perivascular niche i.e. the space between the endothelium and the blood brain barrier. To examine why T-cells remain trapped within this niche in lower grade gliomas, we are performing multiplex immunohistochemical stainings and combine these findings with spatially resolved transcriptomics, bulk and single nucleus sequencing. To ensure these analyses are done in a near isogenetic background, we make use of tumors sampled at multiple timepoints. Tumors progressing to higher grade show increased tumor invasion and we are currently examining which molecular pathways underlie this process.

Keywords: perivascular niche – extravasation – glioma

S6.3

Understanding and targeting the microenvironment in IDH-mutant gliomas

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Isocitrate dehydrogenase 1 (IDH1)-mutant gliomas represent a group of diffusely infiltrating brain tumors that often affect young adults. Mutations in IDH1 result in the increased production of R-2-hydroxyglutarate (R-2-HG) and constitute a distinct, metabolically skewed biological entity. These tumors are associated with less abundant and phenotypically altered immune cell infiltrates compared to IDH1 wild-type tumors. The purpose of our studies is to assess the impact of intratumoral R-2-HG accumulation on immune cells within the glioma microenvironment and its consequences for immunotherapeutic approaches. In a large cohort of human gliomas, T cell abundance in IDH1-mutant tumors was assessed. Transcriptomic, metabolic, and functional analyses of human T cells were applied to assess R-2-HG-dependent perturbation T cell effector functions. Antitumor immunity to experimental murine IDH1-mutant tumors was assessed by inhibition of R-2-HG production. In addition, R-2-HG-exposed tumor-associated macrophages (TAMs) were investigated by integrated single-cell transcriptomic and proteomic analyses of human control and glioblastoma samples and trajectory analyses of antigen-presenting cells were performed. We could show that IDH1-mutated gliomas subdue their adaptive and innate immune microenvironment by prompting a multifaceted reprogramming of myeloid cell metabolism and inhibition of T cell receptor signaling-dependent T cell effector functions. Our findings argue for the development of new therapy concepts that incorporate the cell-specific immunomodulatory tactics of IDH1-mutated gliomas into future efforts of immunotherapy.

Keywords: Glioma – IDH1 – microenvironment – immunotherapy

Symposium 7 TDP-43 proteinopathies

S7.1

Neuropathology of FTLD-TDP

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Frontotemporal lobar degeneration with TDP-43-immunoreactive pathology (FTLD-TDP) is the most common pathological substrate for clinical frontotemporal dementia (FTD) and for amyotrophic lateral sclerosis with dementia. FTLD-TDP can be subclassified based on the type and cortical laminar distribution of neuronal inclusions. The relevance of these pathological subtypes is supported by relatively specific clinical and genetic correlations. Recent evidence suggests that the different patterns of pathology are a reflection of biochemical differences in the pathological TDP-43 species, each of which is influenced by differing genetic factors. As a result, FTLD-TDP subtype may be an important factor to consider when developing clinical biomarkers and targeted therapies for FTD. This review will focus on the pathological features, clinical and genetic correlations of the currently recognized FTLD-TDP subtypes, as well as several novel patterns of TDP-43 pathology.

Keywords: frontotemporal dementia – frontotemporal lobar degeneration – TDP-43

S7.2

Cellular and system vulnerability in ALS

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Amyotrophic Lateral Sclerosis (ALS) is a clinically defined syndrome associated with diverse genetic aetiologies and molecular neuropathologic phenotypes. Strictly defined, it is a syndrome that primarily involves degeneration of upper and lower motoneurons; however, neuropathologically ALS is a disease of neurons and glia affecting motor and non-motor systems of the CNS. Here, I will discuss how neuropathology can inform the nosology of ALS by examining extremes of phenotype and genotype-phenotype relationships, and why this is important in the age of emerging genomic therapies for ALS. Further, I will summarize concepts of selective cell and system vulnerability in ALS, emphasizing the important role modern human brain tissue analytics has in providing scientific leads for reductionist modelling of ALS pathogenesis.

Keywords: ALS – selective vulnerability – TDP-43 – FUS – SOD1

Symposium 8 Myositis – clinical, morphological and differential diagnostic highlights

S8.1

Clinical diagnostic aspects in myositis

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Inflammatory myopathies can be classified into 4 entities: inclusions body myositis (IBM), immune mediated necrotizing myopathies (IMNM), overlap myositis, and dermatomyositis (DM). This classification is based on different clinical, pathological and biological criteria. More and more evidence also shows that the pathophysiology of these different entities is distinct. As far as the clinical aspects of these entities are concerned, there are also major differences between them. IBM is a purely muscular disease without any other systemic damage. The disease usually sets in after 60 years and is most often very slowly progressive. The initially predominant attack of quadriceps and finger flexors is characteristic of this entity. This myositis responds very poorly to conventional immunosuppressive treatments. IMNM are also primarily muscular diseases. They occur at any age, including in children. Muscle damage mainly affects the limb girdles. If this entity generally responds well to conventional immunosuppressive treatments, the functional prognosis in the medium term is bad because this disease leaves important muscle sequelae. The leader of overlap myositis is the antisynthetase syndrome (ASyS) of which besides the myositis leading to a myopathy of limb girdles, includes arthralgia, mechanic hands, Raynaud phenomenon, interstitial lung disease (which makes the prognosis of this syndrome) and sometimes general signs such as fever with inflammatory syndrome. DM in its typical form is easily recognized by the association of limb girdle myopathy and typical skin signs, such as heliotrope rash, Gottron's papule or hands… The DM occurs at all ages of the juvenile forms to the elderly subject then often associated with cancers. Because the therapeutic implications are different between these 4 entities, it is important to know how to distinguish them based primarily on the clinic.

Keywords: myositis – IBM – IMNM – ASyS – DM

S8.2

Morphological diagnostic aspects in myositis

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Myositis is a heterogeneous group of inflammatory muscle diseases affecting all ages and having very different prognostic implications linked to the specific subtype of myositis. In the past, myositis was relatively simply subdivided into dermatomyositis (if the skin was involved) and polymyositis (if the skin was not involved), later IBM was included if vacuoles were present on biopsy. This simplified approach to inflammatory myopathies has been revolutionized by precise morphological and molecular approaches addressing pathophysiological characteristics of the different diseases. Of note, also the autoantibody profiles (so-called myositis-specific autoantibodies), that can be attributed to different types of myositis are of much diagnostic and prognostic relevance. On this basis, the diversity but also unifying aspects of different diseases are described and a focus is laid on prognostic outcomes of the different types of myositis as well as on therapeutic options, which may result from insights into the molecular basis of these diseases.

Keywords: myositis – autoantibodies – IMNM – DM – IBM

S8.3

Differential diagnostic aspects in myositis

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Introduction: With the novel options of myositis specific antibodies for the diagnostics of myositis syndromes, one should be aware that their sensitivity and specificity is not

100%. Thus, if primary immunosuppressive therapy failed, a diagnostic muscle biopsy is still needed, particularly for the differential diagnoses of hereditary myopathies. Several hereditary myopathies are recognized having inflammatory impacts during their disease evolution. Methods and results: As examples, since many decades, the x-chromosomal linked Becker muscular dystrophy, the autosomal dominant facioscapulohumeral muscular dystrophy, several recessive muscular dystrophies may display inflammatory cells. Furthermore, one must be aware of possible double troubles of acquired and hereditary myopathies reflected in one biopsy specimen. Conclusion: This presentation will cover classic findings and help to decipher diagnostic differences.

Keywords: myositis – limb girdle myopathy – triple trouble

Symposium 9 White matter and oligodendrocyte pathology: new insights in neurodevelopmental diseases and epilepsy

S9.1

White matter pathology in vanishing white matter: the role of astroglial pathology

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Introduction: Leukodystrophies are genetic disorders of the brain white matter due to defects in any of its structural components. Amongst these, are the astrocytopathies, disease due to specific genetic defects in astrocyte-expressed protein or in which astrocytes drive the disease mechanisms. Objectives: To highlight the cell-autonomous and non-cell-autonomous mechanisms by which astrocytes cause white matter pathology in the astrocytopathic leukodystrophy Vanishing White Matter. Methods: Brains of 10 vanishing White Matter patients were investigated by means of immunohistochemistry, Western blotting, real-time qPCR and size-exclusion chromatography. Cells of Vanishing White Matter mice were employed in co-culture systems. Results: Astrocytes are dysmorphic, immature and functionally asthenic in Vanishing White Matter, have an abnormal intermediate filament composition and negatively impact on the composition of the extracellular matrix. This hampers oligodendrocyte precursor maturation and prevents proper myelination. Astrocytes in the retina and gastrointestinal tract (enteric glia) are also involved. Conclusion: vanishing White matter is a prototypic astrocytopathic leukodystrophy, in which all aspects of pathology can be driven back to astrocytic dysfunction. Astrocyte thus represent a cellular therapeutic target in this leukodystrophy.

Keywords: White matter – astrocyte – leukodystrophy

S9.2

Myelin loss and oligodendrocyte pathology in tuberous sclerosis and other mTORopathies

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Epilepsy is one of the most common neurological diseases according to the World Health Organization (WHO) affecting around 70 million people worldwide. Patients who suffer from epilepsy also suffer from a variety of neuro-psychiatric co-morbidities which they can experience as crippling as the seizure condition itself. These co-morbidities are commonly associated with abnormal white matter in the brain. Up until now little is known about white matter abnormalities in epilepsy pathology. So far, our studies included patients with pathologic constitutive activation of the highly conserved mechanistic target of rapamycin (mTOR)-pathway, the so-called "mTORopathies" who are found to have epilepsy, cognitive co-morbidities and white matter abnormalities. Imaging studies of tuberous sclerosis complex (TSC) patients indicated that disrupted neuronal microcircuits in combination with underconnectivity of white matter fiber bundles are associated with cognitive failure. A finding that is not exclusively detected in TSC patients but can be identified across many other epilepsy syndromes, proving the relevance of white matter disturbances for cognitive decline in epilepsy patients. Utilizing a combination of comprehensive clinical data and resected brain tissue of patients undergoing epilepsy surgery hypomyelination is a consistent finding in epilepsy pathology, such as the mTORopathies. Previously we showed that myelin – the major component of fast signal transduction in the white matter – is reduced in epileptogenic tissue. More detailed analysis of the white matter components revealed that the loss of myelin integrity is associated with an impaired turn-over and maturation of myelin producing cells (oligodendroglia) caused by two underlying phenomena: the malformative process itself and the micro-environment of the epileptogenic lesion.

Keywords: epilepsy – focal cortical dyplasia – white matter – myelin

S9.3

White matter in temporal lobe epilepsy: clinico-pathological correlates

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Our understanding of the concept of temporal lobe epilepsy (TLE) asso-

ciated with hippocampal sclerosis has recently shifted from a focal epilepsy with a localized pathology to a brain network disorder. This has paradigm change has emerged largely through neuroimaging studies in large patient cohorts with recent emphasis on alterations of the white matter (WM). The ENIGMA-epilepsy consortium study of diffusion weighted imaging (diffusion tensor imaging (DTI)) in 1249 epilepsy patients shows common abnormalities of mean diffusivity/fractional anisotropy (MD/FA) in WM projection pathways, which was pronounced in TLE. Neuroimaging measures of WM pathology with DTI show also promise as a predictors of cognitive decline in TLE. Investigation of the pathological correlates of neuroimaging findings in the WM somewhat lag behind. An earlier MRI study in TLE/HS series showed that grey/white matter blurring and FLAIR/T2-weighted WM hyper-intensities correlated with both loss of myelin and axons in resected samples and poorer memory performance; this is supported by more recent transcriptomics studies in TLE. In the hypomyelination associated with FCD and other mTORopathies, reduction of axons, myelin, altered oligodendrocytes and their progenitor cell densities have been related to seizure duration and also mTOR activity. Likely pathomechanisms for WM alteration in TLE include microstructural tract degeneration along epilepsy networks and plausibly as a secondary effects of epilepsy-related vascular degeneration. Age-accelerated pathology of small vessels in the WM is recognized in surgical resections in TLE, altered capillary and pericyte density and distribution; this could have functional impact on both small vessel hemodynamics and the blood brain barrier, as supported in experimental epilepsy models. Future ongoing research questions regarding the causes and clinical significance of white matter degenerative pathology in TLE will be discussed.

Keywords: white matter temporal lobe epilepsy

Symposium 10 Neuropathology training, courses and examination (EFN) in Europe

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Description of the Session: "Roundtable" discussion, with lecturers and participants of Euro-CNS courses, examiners and successful candidates of EFN examinations. Short presentations with course and exam cases. Advice on how to prepare for a successful EFN exam. Information on how Euro-CNS can support prospective candidates with fellowship programmes, courses, travel grants. Neuropathology training in Europe: overview of the present situation, challenges, opportunities and strategies, with neuropathologists and trainees from European countries.

Symposium 11 Intratumoral heterogeneity

S11.1

Dissecting response to treatment in adult patients with a glioma

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The evolutionary processes that drive universal therapeutic resistance in adult patients with diffuse glioma remain unclear. We established the Glioma Longitudinal Analysis Consortium (GLASS) to define the molecular landscape of glioma evolution and dissect the processes contributing to treatment response. Through mutational and copy number analyses across the three major subtypes of diffuse glioma, we observed that driver genes detected at initial disease were retained at recurrence, while there was little evidence of recurrence-specific gene alterations. Treatment with alkylating-agents resulted in a hypermutator phenotype at different rates across glioma subtypes, and hypermutation was not associated with differences in survival. We found that the clonal architecture of each tumor remains similar over time and that absence of clonal selection was associated with increased survival. Ionizing radiation causes DNA damage and is a mainstay for cancer treatment, but we have limited understanding of its genomic impact. We analyzed mutational spectra following radiotherapy in 190 paired primary and recurrent gliomas and identified radiotherapy-associated significant increases in the burden of small deletions (1 – 20 bp) and large deletions (20+ bp to chromosome-arm length). Small deletions were characterized by a larger span size, lacking breakpoint microhomology and were genomically more dispersed when compared to pre-existing deletions and deletions in non-irradiated tumors. Mutational signature analysis implicated c-NHEJ-mediated DNA damage repair and APOBEC-

mutagenesis following radiotherapy. A high radiation-associated deletion burden was associated with worse clinical outcomes, suggesting that effective repair of radiation-induced DNA damage is detrimental to patient survival. These results may be leveraged to predict sensitivity to radiation therapy in recurrent cancer. Our results collectively argue that the strongest selective pressures occur early during glioma development and that current therapies shape this evolution in a largely stochastic manner.

Keywords: glioma – evolution – radiation – treatment response

S11.2

Epigenomic contribution to glioblastoma heterogeneity

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Glioblastoma is characterized by widespread genetic and transcriptional heterogeneity, yet the role of the epigenome in glioblastoma disease progression is less well understood. Starting from recent work on genome-scale maps of DNA methylation in IDH wildtype glioblastoma, I will introduce bisulfite sequencing as alternative technique to assess DNA methylation patterns, highlight its applicability to routinely collected FFPE samples, and dissect our findings in matched primary and recurring glioblastoma samples. On the basis of these data, we identify subtle differences between primary and recurring tumors, links between DNA methylation and the tumor microenvironment, an association of epigenetic tumor heterogeneity with patient survival, and relevant sex-specific differences. To put the DNA methylation patterns we observed in glioblastoma bulk tumors in a broader epigenomic disease context, I will ultimately highlight differences to DNA methylation patterns of lower grade gliomas and introduce newer single-cell based approaches.

Keywords: glioblastoma – epigenome – disease progression

S11.3

Role of intrinsic tumor plasticity and microenvironment in creating intratumoral heterogeneity in glioblastoma

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Introduction: Phenotypic plasticity recently emerged as a major contributor to intra-tumoral heterogeneity and the development of treatment resistance in solid tumors. Increasing evidence indicates that glioblastoma (GBM) cells display prominent intrinsic plasticity. GBMs create a dynamic ecosystem, where heterogeneous tumor cells interact with the tumor microenvironment to establish different niches. It is currently not clear to what extend the equilibrium of phenotypic states and intra-tumoral heterogeneity depends on intrinsic genetic and epigenetic tumor features as well as extrinsic pressure from the microenvironment and treatment. Methods: Here we examined inter- and intra-tumoral GBM heterogeneity at the single cell transcriptomic and proteomic level in treatment naive and treated GBMs. Tumor cell subpopulations were further classified based on four cell membrane markers (CD133, CD15, A2B5 and CD44). The resulting 16 subpopulations were FACS isolated and functionally analyzed. Mathematical Markov modelling was applied to calculate state transitions between cell states in different microenvironmental conditions. Genome-wide shRNA screen revealed genes essential for adaptation of tumor cells upon invasion. Results: Single cell transcriptomic data show extensive intra-tumoral heterogeneity, where distinct GBM phenotypic states co-exist within GBM-specific microenvironment. Treatment drives entire GBM ecosystem towards resistant states. Functional assessment revealed that all GBM subpopulations carry stem-cell properties and have the capacity to recreate phenotypic heterogeneity. Cellular states appear non-hierarchical, reversible and occur via stochastic state transitions of existing populations, striving towards an equilibrium instructed by the microenvironment and treatment. Conclusion: Phenotypic heterogeneity in GBM results from intrinsic plasticity allowing tumor cells to effectively adapt to new microenvironments and escape treatment. GBM cells with stem cell properties do not represent a clonal entity defined by distinct functional properties and transcriptomic signatures, but a cellular state that is determined by the microenvironment. Novel treatment strategies should tackle the inherent plasticity allowing GBM cells to transit towards treatment resistant states.

Keywords: Glioblastoma – tumor heterogeneity – plasticity – cancer stem cells – microenvironment

S11.4

Brain tumor invasion into the CNS – mechanisms of action

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Background: To get a comprehensive molecular and dynamic picture of glioblastoma (GBM) invasion in real-time, we have used a highly characterized ex-vivo brain organoid (BO) invasion model as a target tissue for human GBM cells. The BOs contain a fully differentiated brain structure. It has been shown that GBM cells have the ability to form synapses with neural cells pointing at

an extensive communication network between brain cells and GBM cells. Through metabolomic studies, we have shown that this communication network can be mediated via the metabolites Glutamine and Glutamate, -both known to play a role in GBM growth. Methods: Perampanel is an antiepileptic agent, that functions as AMPA glutamate receptor antagonist that has been shown to inhibit GBM growth. In order to delineate how Perampanel affects GBM invasion we here utilised a highly characterized or BO invasion model where single cell invasion was studied in real-time following treatment. Results: Perampanel treatment significantly reduced tumor cell invasion into the brain organoids with the strongest effect seen towards single cell invasion into the brain parenchyma. Here, single tumor cell invasion ratio was reduced by 72% in compared to the control group ($p = 0.0033$). In contrast, collective cell invasion was reduced by 19% ($p = 0.028$). Conclusion: Perampanel significantly inhibits GBM invasion, suggesting an important role of the glutamate-glutamine cycle between the GBM cells and neurons in the invasion process. This communication seems to be more prominent where single GBM cells invade into the brain parenchyma compared to areas where collective invasion take place.

Keywords: glioblastoma – invasion – Perampanel

Symposium 12 Dynamic aspects of amyloid-β

S12.1

The relationship between spreading and maturation of amyloid-β pathology in AD

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Background: Spreading of AD-related Aβ deposition follows a hierarchical pattern, in which different brain regions become step-by-step involved. Seeds can induce Aβ deposition and probably drive its propagation. A sequential pattern starting with deposition of presumably non-modified Aβ, followed by AβN3pE and AβpSer8 characterizes Aβ aggregate maturation in the neocortex. Objectives: 1. To clarify whether propagation of Aβ pathology into secondarily affected brain regions follows a similar sequence as observed in the neocortex. 2. To analyze the impact of seeds and their composition on propagation and initiation of Aβ deposition. Methods: Biochemical and histopathological analysis of Aβ plaque composition with antibodies against Aβ, AβN3pE, and AβpSer8 in the neocortex, cingulate gyrus, basal ganglia, and cerebellum of 38 human autopsy cases. Injection of human brain-derived Aβ seeds from brains in early and late maturation stages of Aβ pathology into the hippocampus of 2-month-old APP23 mice and subsequent histopathological investigation at 6 months. Results: In all investigated human brain regions the deposition of Aβ detectable with antibodies against non-modified forms of Aβ preceded that of AβN3pE followed by AβpSer8. Injection of seeds into APP23 mouse brain induced Aβ deposits positive for all different modified and non-modified forms of Aβ at 6 months of age regardless of the composition of the seeds. Conclusion: Propagation of Aβ plaque pathology in the human brain is characterized by Aβ aggregate maturation in each newly affected region, as observed in the neocortex. This process can be accelerated by Aβ seeds regardless of the composition of the seeds.

Keywords: Alzheimer's disease – spreading – maturation – amyloid

S12.2

Evidence for the person-to-person transmissibility of amyloid-β

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Introduction: Experimental seeding of Aβ has been demonstrated in animal models. Growing evidence demonstrates that Aβ transmission has occurred through medical procedures in humans, such as contaminated growth hormone administration and dura mater grafting. Objectives: Here we show that Aβ seeds may be transmissible through neurosurgery and in some patients with iatrogenic Aβ, also Alzheimer's-type tau pathology is observed in the neocortex. Material and methods: The pathology archive was searched for young adults with histologically confirmed CAA and a control group of 50 consecutive age-matched patients. Aβ and tau pathology was assessed histologically and previous medical interventions were identified from the clinical notes. In all patients, the APP, PSEN1, PSEN2 were screened and APOE polymorphism determined. Literature was searched to identify further patients with young-onset CAA. Results: Four patients with CAA and past history of neurosurgery were identified. Three had undergone diagnostic brain biopsy for investigation of intracerebral hemorrhage and one had died of complications from intracerebral hemorrhage. All had undergone neurosurgery several decades earlier for various reasons. None had mutations in genes associated with early Aβ pathology. One patient had definitely not received a cadaver-derived

dura mater graft, but this information could not be traced in the other three. In the literature, we identified four patients with CAA and a history of childhood neurosurgery. Aβ pathology was not observed in the selected control group. Subsequently, we have identified three additional patients with iatrogenic Aβ pathology and significant Alzheimer's type tau pathology in the cortical brain biopsies. Conclusion: Over the last six years, strong evidence has been gathered indicating that Aβ is transmissible in humans through medical procedures, involving human cadaver-derived tissues and contaminated surgical instruments. Our recent observation shows that following incubation periods exceeding three decades also significant neocortical tau pathology develops in some of the patients.

Keywords: Cerebral amyloid angiopathy – Alzheimer's disease – iatrogenic transmission – transmissible proteinopathy – cadaver-derived dura mater

S12.3

Removal of amyloid-β from the brain by immunotherapy

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Introduction: Abnormal accumulation of proteins in the CNS is a unifying feature of neurodegenerative diseases. Examples include amyloid-β (Aβ) and hyperphosphorylated tau in Alzheimer's disease (AD). It is tempting to view the accumulation of such proteins, once initiated, as inevitably progressive. Objectives: To explore the effects of Aβ immunotherapy on Aβ and tau. Method: We performed a 15 year neuropathological follow up of 22 patients in the first trial of Aβ immunotherapy for AD. Results: More than 80% of patients with AD had evidence of Aβ removal, the extent of which varied considerably. Persistent effects of immunization were still present after 14 years in some cases. Tau aggregates were reduced in areas of cerebral cortex from which Aβ had been removed, but spread of tau through the brain may have been unaffected. Microglial activation was also reduced in the long term. Conclusion: These studies indicate that Aβ aggregation is a dynamic process, amenable to reversal. Local reduction in tau, where Aβ had been removed, supports the concept that tau pathology is downstream of Aβ, consistent with the amyloid cascade hypothesis. However, the likely continued spread of tau through the brain implies that treatment of established AD requires additional targeting of tau. The degree to which removal of neurodegeneration-associated protein aggregates is functionally beneficial remains to be clarified. Active immunization against Aβ prior to disease onset may be more beneficial.

Keywords: Alzheimer's disease – immunotherapy – amyloid-β – tau

Symposium 13 COVID-19 and neuropathology

S13.1

Brain pathology of COVID-19

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Coronavirus disease 2019 (COVID-19) is caused by the infection with SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). SARS-CoV-2 affects multiple organ systems including the central nervous system (CNS) where it leads to CNS dysfunction (Neuro COVID-19). Mechanisms of SARS-CoV-2 neuropathogenesis including the targeting of the brain and elucidation of the sequence of events underlying CNS damage are only poorly understood. We and others have investigated this by neuropathological deep phenotyping using morphological methods and molecular neuropathology in representative cohorts of patients dying from/with COVID-19 and in adequate control cohorts. Our studies show that Neuro COVID-19 is characterized by a compartmentalized and region-specific perivascular glial and neuroinflammatory response with activation of microglia which is found in nearly all patients dying from/with COVID-19. Conversely, SARS-CoV-2 and SARS-CoV-2 viral proteins can only be found in low amounts in a subset of COVID-19 patients' brains. In the presentation, it will be illustrated how neuropathological deep phenotyping can contribute to elucidate disease mechanisms in Neuro COVID-19.

Keywords: Coronavirus – SARS-CoV-2 – NeuroCOVID-19 – COVID-19

S13.2

COVID-19 encephalitis: the pathological evidence

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Introduction: SARS-COV2 may cause a wide range of neurological manifestations in about 30% of covid 19 patients. Cases of encephalitis have been described in patients presented with severe respiratory distress with estimated incidence of 58/100,000 patients. However, the correlated pathological changes of these cases of encephalitis and its

pathogenesis are poorly understood. Methods: We have examined brains from 23 randomly referred cases of patients dying with COVID-19, with spectrum of neuropathological findings such as hypoxic-ischemic changes, infarcts, hemorrhages and necrotizing encephalopathy and mild to moderate increase in number of activated microglia cells. There are 5 cases of intense infiltration of brainstem structures with microglia cells and mild T lymphocyte infiltration which raises the possibility of what is called "brainstem encephalitis" although with poorly correlated neurological manifestations. In these cases, there is intense activation of microglia cells with microglia nodules formation and mild infiltration with T lymphocytes but there is no significant demyelination, necrosis or axonal damage. The immunohistochemistry and in-situ hybridization failed to demonstrate SARS-COV2 virus or other viruses. On the other hand, we found only mild increase in number of activated microglia cells but without microglial nodules in the brainstem of 3/6 patients that died from non-COVID related septicemia and multi-organ failure. Results: These results suggest that the inflammatory changes in the brainstem structures are most likely related to peculiar substantial systemic inflammatory reaction and massive release of cytokines (cytokines storm) in COVID-19 patient causing activation of innate immunity and alteration of blood brain barrier (BBB) in brainstem structures. Therefore, this pathology should be termed as "COVID-19 encephalopathy" rather than encephalitis. Conclusion: This and other works highlighted the importance for more research into the role of microglia cells in brain pathology in acute and long Covid19 diseases including psychiatric manifestations.

Keywords: COVID-19 encephalitis

Workshops

Workshop 1
Primary tauopathies

W1.1

Overview of tau pathologies

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Filamentous deposits of known composition in brain cells define most human neurodegenerative conditions. Assemblies of the microtubule-associated protein Tau comprise the most frequent neurodegenerative proteinopathies. Diseases with filamentous Tau pathology can be divided into three groups, based on the isoforms in the filaments. In these diseases, be they sporadic or inherited, Tau is extensively modified post-translationally. Conditions with pathological Tau accumulation are frequently referred to as tauopathies. Tauopathies are morphologically, biochemically and genetically heterogenous diseases with abundant Tau inclusions in many different anatomical distribution patterns. The purpose of this presentation is first, to demonstrate morphologies detected by the generally used anti-phospho-Tau antibody AT8 in the human brain; second, to guide on the interpretation of the constellation of tau immunoreactivities as tau pathology, and third, to interpret the anatomical constellation of tau pathologies as a disease entity or major tauopathy. A careful approach on the examination of histological features of tissue lesions and tau-immunoreactivities focusing on cells combined with the anatomic distribution patterns allows the diagnosis of major tauopathies and distinguishing these from rare forms. The value of this approach is to allow those working on Tau-related neurodegenerative conditions to communicate their findings in a concise and unambiguous fashion.

Keywords: Neurodegeneration – proteinopathy – tau – tauopathy

W1.2

Tau pathology related to brain trauma

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The role of traumatic brain injury as a risk factor for future neurodegenerative disease has been an area of active research for almost a century. Different risk factors appear to be associated with a single impact and multiple mild episodes of mild TBI. A novel tauopathy, originally known as dementia pugilistica but now called chronic traumatic encephalopathy (CTE) appears to be a neuropathological marker of repeated mild TBI. CTE shows a unique distribution of tau pathology when compared with other tauopathies, although consideration needs to be given to alternative diagnoses such as ARTAG. Recent data provide novel insight into the ultrastructure of tau in CTE, and the incidence of CTE as a co-pathology with other neurodegenerative disorders. However, to date, CTE remains a neuropathological diagnosis in search of a clinical phenotype. This talk will cover the evolution of this unique tauopathy and discuss diagnostic approaches, including grading. It will consider the clinical phenotype and look at future research.

Keywords: CTE – TBI – tauopathy

Workshop 2 Muscle biopsy and molecular biology: A successful cooperation

Chairs: Martin Lammens[1], Werner Stenzel[2]

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Session description: Is there still a place for neuropathological examination of muscle biopsies, especially in probably genetical neuromuscular disorders? In this workshop, several cases will be discussed in which the complementarity of molecular biological findings and biopsy findings in muscle biopsies will be demonstrated.

W2.3

Differential diagnosis of vacuolar myopathies in the NGS era

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Introduction: Altered autophagy accompanied by abnormal autophagic vacuoles is a common denominator of many familial and sporadic non-inflammatory muscle diseases. Even in the era of next generation sequencing (NGS), late-onset vacuolar myopathies remain a diagnostic challenge. Methods: We studied 32 adult patients with the histological diagnosis of vacuolar myopathy. We established a molecular genetic diagnosis in 17 patients. Results: Pathogenic mutations were found in genes typically linked to vacuolar myopathy (GNE, LDB3/ZASP, MYOT, DES, TRIM32, GAA), but also in genes not regularly associated with severely altered autophagy (FKRP, DYSF, CAV3, COL6A2, GYG1, FSHD2). Characteristic histopathological features including distinct patterns of myofibrillar disarray and evidence of exocytosis helped to distinguish causes of vacuolar myopathy. Biopsy validated the pathogenicity of the novel mutations p.(Phe55*) and p.(Arg216*) in GYG1 and of the p.(Leu156Pro) TRIM32 mutation combined with compound heterozygous deletion of exon 2 of TRIM32 and expanded the phenotype of Ala93Thr-caveolinopathy and of LGMD2i due to FKRP mutation. In 15 patients no causal variants were detected by Sanger sequencing and NGS panel analysis. In 12 of these, WES was performed, but did not yield any definite mutation or likely candidate gene. In one patient with familial muscle weakness, the vacuolar myopathy was eventually linked to chloroquine therapy. Conclusion: Our study illustrates the wide phenotypic and genotypic heterogeneity of vacuolar myopathies and validates the role of histopathology in assessing the pathogenicity of novel mutations detected by NGS. In a sizable portion of vacuolar myopathy cases, it remains to be shown whether the cause is hereditary or degenerative.
Keywords: Vacuolar myopathy – autophagy – next generation sequencing (NGS) – myofibrillar myopathy – sarcotubular myopathy

Workshop 3 Pituitary and sellar lesions

W3.1

The 2017 WHO classification of pituitary neuroendocrine neoplasms. What have we learnt?

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The current classification of pituitary neuroendocrine tumors according to the pituitary cell lineages is based on the immunohistochemical expression of anterior pituitary hormones and pituitary specific transcription factors. The three main groups of pituitary tumors have been defined: Pit-1 positive tumors including somatotroph, lactotroph, thyrotroph, and plurihormonal Pit-1 tumors, T-Pit positive corticotroph tumors, and SF-1 positive gonadotroph tumors. Within each group, hormone-producing and clinically nonfunctioning tumors occur. Null cell adenomas, defined as non-functioning tumors negative for both pituitary hormones and transcription factors, have been reduced to less than 2% of all pituitary tumors. The previous designation "atypical adenoma" has been replaced by "high-risk adenoma" and the latter does not provide more precise criteria to predict tumor invasiveness and risk of recurrence. Several histological subtypes have been characterized as potentially more aggressive, although the data supporting their aggressiveness may not always be convincing. Challenges related to the next WHO classification are adoption of the term "pituitary neuroendocrine tumor", revision of some controversial tumor categories, such as "null cell adenoma" and plurihormonal Pit-1 tumor, and integration of the recent molecular genetic data. Future molecular genetic studies may improve the classification of pituitary neuroendocrine tumors, expand the panel of prognostic and predictive markers in pituitary

neuroendocrine tumors and direct the search for novel therapeutic targets.

Keywords: pituitary adenomas – pituitary neuroendocrine tumors

W3.2

Morphology meets genomics and epigenomics: should we move towards an integrated tissue diagnosis of PitNETs?

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The nosology of pituitary adenomas/pituitary neuroendocrine tumors (PitNETs) is evolving. To date molecular pathological data are not integrated into any WHO classification system. Unlike in other neoplasms, recurrent point mutations are not very common in PitNETs. However, emerging data suggest that somatic mutations in GNAS, USP8, SDH or ATRX may segregate with certain clinicopathological features, which may be clinically relevant. Structural variations such as copy number variants (CNVs) and epigenomic changes may provide an additional layer of stratification within histologically defined PitNETs. A complex picture is emerging, which will be discussed using somatotroph PitNETs as an example. Integration of these and other datasets has been attempted in one seminal study, which has led to some unexpected findings relating to the blurring of transcription-factor defined PitNET lineages. Finally, it is proposed that future iterations of PitNET classification systems may benefit from adoption of the concept of integrated histomolecular diagnostics.

Keywords: Pituitary adenoma – PitNET – epigenomics – transcription factor

W3.3

Molecular pathways and targets in pituitary tumors

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Pituitary tumors are monoclonal neoplasms that are in their majority sporadic and are only rarely found as part of genetic syndromes. Oncogenes like HRAS and BRAF are rarely mutated in sporadic pituitary tumors, while TP53 mutations are limited to aggressive cases. Recurrent somatic mutations in GNAS, USP8 and in lesser extent USP48 are present in almost half of somatotroph and corticotroph tumors respectively. Oncogenes frequently mutated in other cancers, such as, HRAS and BRAF are rarely mutated in sporadic pituitary tumors, while TP53 mutations are limited to aggressive cases. No other driver mutations have been identified for the majority of sporadic pituitary tumors, indicating the presence of other, yet to be discovered, genetic defects. The pathophysiology of pituitary tumors has been tightly linked to aberrant hypothalamic, peripheral hormonal as well as autocrine/paracrine signals. Posttranscriptional/ posttranslational deregulations in factors involved in cell cycle regulation and growth factor signaling are commonly found in pituitary tumors. In the last years, the use of pangenomic techniques has advanced our understanding of the molecular mechanisms underlying pituitary tumorigenesis. In depth analysis of these data is expected to enhance our understanding on the mechanisms driving pituitary tumor progression and reveal drugable targets for the improved management of these neoplasms.

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Keywords: Pituitary tumor – pathogenesis – molecular pathways – molecular – targets

W3.4

Pituitary lesions from an endocrinologist's perspective

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Large pituitary lesions may be diagnosed due to mass effect, typically visual field impairment, but also hyper- or hypo-secretion of pituitary hormones may give us the first sign or symptom. However, an increasing number of patients are referred to a Department of Endocrinology due to a pituitary incidentaloma. Our detailed knowledge on the incidence and prevalence of specific pituitary lesions is based on cases, where surgery has been performed, enabling pathological characterization. Pituitary adenomas are the most frequent pituitary lesion. Especially, clinically non-functioning pituitary adenomas have a wide range of growth potential. Most of these adenomas are silent gonadotroph adenomas; however, adenomas like clinically silent ACTH or GH and plurihormonal Pit-1-positive tumors are also diagnosed. We need to be aware of changes over time in the clinical presentation of a pituitary adenoma, where a silent adenoma may become a clinically secreting adenoma and a patient with hyperprolactinemia may develop acromegaly. The 2017 World Health Organization (WHO) classification system on the pituitary is based on tumor cell lineage and transcriptional factor nuclear staining. If this classification was fully implemented in all pituitary centers, it would help the endocrinologist in planning the individual treatment and follow-up. Sometimes we need no more than a scan of the pituitary region to decide, that the tumor is aggressive, however, in selected cases, it is necessary to assess tumor proliferative potential by mitotic count and Ki-67 index. Syndromic and inherited forms of pituitary tumors only comprise a small number of pituitary lesions; however, it is helpful for the clinician to have access to this information. Experimental medical therapy, e.g., temozolomide, is indicated in few patients with a pituitary tumor, and we need O6-methylguanine-DNA methyl

transferase (MGMT) status for the clinical decision making. The close collaboration between pathologist and endocrinologist is very fruitful.

Keywords: Pituitary lesions – endocrinologist – syndromic – inherited – adenoma – temozolomide

Workshop 4

Slide seminar on human prion diseases: histotyping and identification of atypical phenotypes

Chairs: Ellen Gelpi[1], Piero Parchi[2,3]

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Session description: Detailed neuropathological examination of human prion diseases has identified reproducible patterns of pathology concerning the topographical distribution of spongiform change and gliosis and the type of prion protein deposits. These histological patterns correlate well with clinical phenotypes, ancillary tests and molecular background. Objectives and methods: In this workshop we will present the different subtypes of human prion disease and will perform the histotyping in an interactive way with the audience. This should help to get particular clues that might be useful to identify subtype-specific patterns.

Workshop 5

Case discussions of CNS tumors with multi-layered information

Chairs: Felix Sahm[1], David Capper[2]

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Session description: The workshop will cover basic principles of next-generation-sequencing and DNA methylation profiling from a technical and diagnostic perspective. Participants will have the opportunity to discuss specific cases in order to select the appropriate work-up, and to come to integrated diagnoses based on the data.

Workshop 6 Developmental neuropathology: recent advances and future challenges

W6.1

The value of postmortem examination in neurodevelopmental diseases

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The birth prevalence of structural brain malformations is around 1 to 2 : 1,000 and increases with the performance of imaging technologies. The most common CNS malformations are microcephaly, hydrocephaly/ventriculomegaly, macrocephaly, myelomeningocele, anencephaly, encephalocele and posterior fossa anomalies. Developmental brain abnormalities can have multiple genetic and non-genetic etiologies including environmental factors, teratogens and vascular insults. A multidisciplinary approach is essential for evaluation and management of affected pregnancies and newborns, and recurrence risk counseling. The diagnosis takes into account pregnancy history, fetal conditions, imaging, postnatal clinical history, laboratory findings, karyotype or better Array-CGH and when necessary, genetic investigations already performed. Neuropathological examination reveals major information about the progression of normal or abnormal development. Most of the time, It leads to the identification of the key lesions allowing the classification of the malformation notably the distinction between acquired/environmental and genetic diseases to guide specific investigations. The distinction between malformations and disruptions is important to provide insights into the pathogenesis, the recurrence risk and genetic counseling. Moreover, some malformations can be caused by genetic or environmental factors, such as holoprosencephaly or polymicrogyria. Advances in high-throughput DNA sequencing have enabled the rapid expansion of genetic test content, resulting in dramatically increased numbers of DNA variants identified. Neuropathological analysis and "phenotype–genotype correlation" are indispensable for investigating the mechanism of gene function, the spectrum of variability, and efficient assessment of variants for pathogenicity determination. The identification of the pathophysiology and molecular pathways that could be targeted by pharmacological agents and neuroprotective strategies also remain an important challenge. A significant number of CNS malformations are still of unknown cause. Collaborative research efforts of clinical teams and researchers are thus a high priority to identify the etiology of the malformations, to understand their pathophysiology, and further development of preventive and effective therapeutic strategies.

Keywords: developmental – neuropathology – malformation – fetal brain

W6.2

Interneurons in cerebral cortical developmental disorders

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Cortical Interneuron dysfunction has been linked to a variety of neurodevelopmental disorders including childhood epilepsies, autism spectrum disorder, intellectual disabilities, and several psychiatric disorders. Understanding interneuron development provides novel insights into the pathogenesis of these diseases. Work over the last decade in my laboratory has focused on understanding the role different molecular pathways play in the development of interneurons (inhibitory neurons) and projection (excitatory) neurons. Through this work we have gained a greater understanding as to how transcription factors influence the proliferation, migration and differentiation of unique progenitor pools. Furthermore, by comparing phenotypic characteristics of different patients, we have learned that interneuron and projection neurons have different metabolic requirements for several aspects of development including cell migration. Thus, we have found that metabolic dysfunction also results in unique interneuron defects, contributing to neurodevelopmental phenotypes. In this workshop the fundamentals of how transcriptional regulation and metabolism participate in interneuron development will be covered as well as how defects play a role in the pathogenesis of neurodevelopmental disorders.

Keywords: Interneurons – transcription factors – metabolism – neurodevelopmental disorder

W6.3

Neuropathology of focal cortical dysplasias: update 2021

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Background: Focal Cortical Dysplasia (FCD) is the most common cause of drug-resistant focal epilepsy in children and young adults. The diagnosis of currently defined FCD subtypes relies on a histopathological assessment of surgical brain tissue using the international ILAE consensus scheme from 2011. Objectives: A new Task Force from the International League against Epilepsy (ILAE) was invited to identify areas of diagnostic challenges in this widely used classification scheme. Methods: The Task Force developed an iterative histopathological agreement trial with genetic testing. Twenty neuropathologists from 15 countries reviewed digital slides from 22 epilepsy patients operated for clinically suspicious FCD. Five genetic labs independently performed panel sequencing of FCD relevant genes in paired brain and blood samples from the same 22 patients. Results: Histopathology agreement based solely on H&E stainings was low in round 1, and gradually increased when using a panel of immunostainings in round 2 and the Delphi consensus method in round 3. Interobserver agreement was good in round 4, when the results of genetic tests were disclosed, i.e. MTOR, AKT3, DEPDC5, NPRL3 and SLC35A2. Conclusion: the diagnoses of FCD 1 and 3 subtypes remained most challenging and were often difficult to differentiate from a normal homotypic or heterotypic cortical architecture. Our results suggest that the current ILAE classification scheme should recognize immunohistochemical stainings for a better histopathological work-up and definition of FCD subtypes. We also propose to add the level of genetic findings to obtain a comprehensive, reliable and integrative genotype-phenotype diagnosis in the near future.

Keywords: Brain – epilepsy – seizure – malformation

Workshop 7 Assessment of the contributions of mixed pathologies in the ageing brain

W7.1

Mixed pathology in the aged demented

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The defining neuropathological features of age associated neurodegenerative diseases are aggregations of misfolded proteins and the neuropathological diagnosis is based on the semiquantitative assessment of these misfolded proteins that constitute the neuropathological hallmark lesion for the respective disease: e.g., Alzheimer's disease (AD), amyloid-β (Aβ) hyperphosphorylated tau (tau); Lewy body diseases, α-synuclein (α-syn); frontotemporal lobar degeneration, tau or TDP-43 or ubiquitin or FUS. In addition, cerebrovascular lesions are assessed for the diagnosis of cerebrovascular disease. However, in brains of elderly patients suffering from neurodegenerative diseases multiple pathologies are usually present and various amounts of neurodegenerative and cerebrovascular pathology are frequently seen even in brains of non-demented elderly. It does indeed become increasingly clear that the clinical picture of dementia in most aged patients results from a multimorbid condition rather than from one single disease. Importantly, this cerebral multimorbididty is generally not detected clinically, which has a detrimental impact on clinical studies since apparently homogeneous study cohorts (e.g., AD)

are likely to be heterogeneous (e.g., AD only, AD and α-syn, AD and TDP-43), which in turn introduces a bias into therapeutic trials and biomarker/imaging studies. Hence, clinical studies should ideally involve neuropathological post mortem assessment to correlate clinical with neuropathological data as this will enable a more accurate stratification of clinical cohorts according to the presence of multiple pathologies. This is highly important in order to interpret clinical data on biomarkers and therapeutic effects.

Keywords: Alzheimer – Dementia – Lewy bodies – TDP-43

W7.2

Amygdala, a hotspot of pathology in the aged

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Introduction: Amygdala (AC) is part of limbic system located to the base of the forebrain. AC receives input from regions such as olfactory bulb, hypothalamus, hippocampus, sensory cortex and ventral tegmental area. Efferent pathways include nuclei of stria terminalis, vagus, nucleus accumbens, and thalamus. Aging-related altered proteins such as hyperphosphorylated tau (HPtau), β-amyloid (Aβ), α-synuclein (αS) and phosphorylated transactive DNA binding protein 43 (pTDP43) are frequently observed within this brain-region. Methods: Aging-related altered proteins were assessed in AC applying immunohistochemistry. The autopsy cohort included 1209 subjects, mean age at death was 75.6 ± (SE) 0.3 whereas 257 (21%) had displayed cognitive impairment (CI) during life. Results: Neuronal HPtau was observed in 63%, glial HPtau in 34%, Aβ in 43%, pTDP43in 32%, and αS in 16% of all. The incidence of all altered proteins increased with age. All four altered proteins were seen concomitantly seen in AC in 5% and three of them in 19% of the subjects. When all four proteins were observed in AC, 58% of subjects displayed cognitive impairment. Conclusion: Altered proteins are indeed common in amygdala. Only 26% of all in our cohort lacked any pathology in AC, most being younger (92% in the youngest and 2% in the oldest group). Only 4% of all demented lacked any protein alterations in AC. Based on the above AC is indeed a hotspot regarding altered proteins and thus symptoms related to functional disturbance of AC should be quite common in all aging-related proteinopathies.

Keywords: Amygdala – tau – aging

Workshop 8 Progress in the pathological diagnosis of pediatric and adult CNS tumors

W8.1

Molecular pathology of epilepsy-related low-grade lesions

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Epilepsy is the commonest chronic neurological disease of childhood and one of the commonest in adults. Some patients have severe treatment-resistant seizures that are caused by focal structural abnormalities. Such patients often require surgical resection to treat their seizures. The most frequent underlying causes are low-grade developmental lesions. For example, in children, malformations are the most common cause amongst those having epilepsy surgery, and in both adults and children, low-grade developmental tumors are the second most frequent. Not only do these patients have severe poorly controlled epilepsy necessitating surgical resection, but many also have significant neurodevelopmental or cognitive problems. Therefore, these diseases are responsible for a substantial burden of disability, both in children and adults, and for which we have poor diagnostic tools and limited therapeutic options. Compared to high grade tumors, our understanding of the underlying molecular and cellular mechanisms of these diseases is relatively limited. They offer not only a significant clinical challenge but also a unique exemplar of the interactions between oncogenic pathways and developmental processes. However, studies of the diseases are hampered by their rarity, their cellular complexity, the poor correlation between molecular and histological classifications, and poor interobserver consistency in their diagnosis. In this talk, I review this data, and summarize some of the underlying cellular mechanisms present in these disorders.

Keywords: Epilepsy – ganglioglioma – dysembryoplastic neuroepithelial tumor

W8.2

Modern diagnostics of ependymal tumors

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Background: The markedly different clinical behaviors of ependymomas are based at least in part to distinct genetic alterations that occur in some groups of ependymomas depending on their location and the age of the patients. Therefore, the WHO 2021 has suggested to classify ependymomas according to their location and underlying genetic alteration. Results: the WHO 2021 recognizes the following types: Supratentorial ependymoma Supratentorial ependymoma, ZFTA-fusion positive Supratentorial ependymoma, YAP1-fusion positive Posterior fossa ependymoma Posterior fossa ependymoma, Group

PFA Posterior fossa ependymoma, Group PFB Spinal ependymoma Spinal ependymomas, MYCN-amplified Myxopapillary ependymomas Subependymoma Therefore, in addition to search for characteristic pathological features to recognize a brain tumor as an ependymoma, pathologists should perform additional techniques to classify them. For intracranial ependymomas, immunohistochemistry offers an invaluable help: anti-65-NFKappaB for supratentorial ependymomas and anti-H3K27Me3 (and/or EZHIP) for infratentorial ependymomas. Confirmation of a presumptive histomolecular diagnosis should be done by appropriate molecular techniques. New fusion-type, not included yet in the WHO classification have been reported. In spinal ependymomas that demonstrate anaplasia, search for MYCN amplification is encouraged. Subependymomas are not stratified according to location. At last, grade is still a matter of debate whereas a grade 2 is now assigned to myxopapillary ependymomas. Conclusion: in 2021 pathologists should provide a histomolecular diagnosis for ependymomas. Papillary, tanicytic, clear cells and anaplastic ependymomas are no longer recognized as entities. DNA-methylation profiling offers an invaluable help to classify these tumors and might be used as a surrogate marker for genetic events. NOS and NEC terminology are still on record.

Keywords: Ependymoma – location – genetic alteration – WHO 2021

W8.3

Differential diagnostics of primitive "embryonal" tumors

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In the last decade, genome-wide sequencing and epigenetic profiling has significantly increased our knowledge on distinct tumor entities in the family of CNS "embryonal tumors". They are mostly composed of immature cells in H&E histology; the identification of specific pathobiological features and characteristic genetic alterations helps to assign such tumors to a specific diagnosis. According to the WHO classification of CNS tumors 2021, the family of CNS embryonal tumors consists of the histologically and geneticallly defined medulloblastoma types, atypical teratoid/rhabdoid tumors, cribriform neuroepithelial tumors (CRINET), embryonal tumors with multi-layered rosettes (ETMR), CNS neuroblastoma, FOXR2 activated, CNS tumors with BCOR internal tandem duplication, and CNS embryonal tumors, NOS. Embryonal tumors can lack typical histological features such as rhabdoid components in ATRT or multi-layered rosettes in ETMR. In addition, they can be misdiagnosed as other (non-embryonal) tumor types with similar morphological appearance but largely divergent clinical and biological behavior. With today's standards of clinical neuropathological diagnostics including immunohistochemical and molecular pathological assays such tumors can be correctly diagnosed. Specific antibodies are well established to lead to the correct diagnosis and exclude histological mimics such as undifferentiated high grade gliomas. In a stepwise algorithm, immunohistochemistry for vimentin, synaptophysin and Olig2 helped to broadly classify embryonal tumors. For example, CNS neuroblastoma was found Olig2 and synatophysin positive but mostly negative for vimentin, in contrast to gliomas that expressed vimentin. In addition, most embryonal entities express characteristic (although not absolutely specific) antigens (e.g. LIN28 in ETMR, BCOR in BCOR-ITD, TTF1 in CNS-neuroblastoma, EMA in AT/RT and CRINET, OTX2 in non-SHH medulloblastomas. Individual molecular assays can be applied to confirm the diagnosis (e.g. PCR for BCOR-ITD, FISH for C19MC in ETMR). In unresolved cases, more extensive sequencing approaches or epigenetic profiling as additional layers of information can help to clarify the diagnosis.

Keywords: Primitive neuroectodermal tumors – embryonal tumors – differential diagnosis – CNS neuroblastoma – ETMR

Workshop 9
B cells in inflammatory demyelinating diseases

W9.1

B-cell inflammation in MS

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Introduction: In MS, B-cell tolerance checkpoints are defective, leading to an accumulation of pathogenic, naive B cells in the blood. Moreover, B cell-rich follicle-like structures have been found in the meninges of MS patients, which are associated with a poor clinical outcome. Which and how B-cell subsets are induced to enter the CNS and contribute to local pathology is underexplored. Objective: In our work, we aim to uncover how peripheral cues trigger the development and effector function of CNS-infiltrating B cells and whether

this coincides with the course of MS and MS-like diseases. Methods: Phenotypes of distinct naive and memory B-cell subsets are analyzed in the blood from treatment-naive and treated MS patients, as well as in post-mortem MS brain tissues. Functional aspects such as class-switching, CNS infiltration and antibody-secreting cell formation are also assessed in vitro (Tfh-like differentiation, brain endothelial transmigration assays). Results: CXCR3-expressing B cells were enriched in the CSF, meninges and white matter from MS donors. These subsets accumulated in the blood from MS patients treated with natalizumab, corresponding to their preferential migration capacity in vitro. Naive B cells of MS patients were more sensitive to differentiate into IgG-secreting cells in IFN-γ-containing cultures. The presence of coding MS risk allele IFNGR2 augmented this sensitivity to IFN-γ. Phosphorylated STAT1 was constitutively expressed in EBV-infected B-LCL carrying the IFNGR2 risk allele. T-bet was further upregulated through additional TLR9 ligation, resulting in enhanced switching. In bone marrow-transplanted MS patients, EBV copy numbers positively correlated with CXCR3 expression in class-switched B cells. In natalizumab-treated MS patients with high EBV load, CXCR3 high memory populations preferentially developed into IgG-secreting cells in vitro. Conclusion: Our data implicate that IFN-γ together with infectious triggers such as EBV potentiate CXCR3(T-bet) + memory B cells to infiltrate the CNS and locally differentiate into IgG-secreting cells in MS.

Keywords: Lymphocytes – development – tissue homing – function – MS

W9.2

Roles of B-cells in MS and potential consequences for therapy

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Elucidating how B cells contribute to the spectrum of CNS inflammatory demyelinating disease, has become of major interest. In part, this interest has been fueled by the success of B cell depleting therapy in limiting multiple sclerosis (MS) relapses. Additional reasons, however, include the growing recognition that B cells may contribute to CNS inflammatory conditions through multiple distinct mechanisms which may, in turn, contribute the pathophysiologic heterogeneity that appears to exist across the disease spectrum. We will provide a brief overview of the range of B cell responses that may participate in CNS inflammatory disease, highlight some of the non-antibody dependent mechanisms underlying B cell involvement, including how B cells may shape T cell and myeloid cell responses in ways that are relevant to CNS inflammation.

Keywords: B cells – antibody-independent functions – cellular interactions

W9.3

Antibody mediated autoimmune diseases of the nervous system

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There has been a major progress in our understanding of the pathogenetic and pathological aspects of antibody mediated autoimmune diseases of the central nervous system. Neuropathological investigations have led to significant advances in our understanding of antibody-associated demyelination, provided important insights into the pathomechanisms of anti-neuronal autoimmune encephalitis, and demonstrated a novel link between inflammation and neurodegnereation. In this presentation, I will review the pathological spectrum of aquaporin4 (AQP4)-antibody positive neuromyelitis optica spectrum disorders (NMOSD) and myelin oligodendrocyte glycoprotein associated disorders (MOGAD) and discuss potential mechanisms that are involved in the formation of demyelinating lesions. Moreover, I will present neuropathological features of different subtypes of anti-neuronal autoimmune encephalitis and discuss the respective inflammatory pathways that may lead to synaptic dysfuntion and neuronal loss.

Keywords: aquaporin-4 – MOG – NMDAR – demyelination – autoimmune encephalitis

W9.4

MOG encephalomyelitis and NMOSD – insights from animal models

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Introduction: Neuromyelitis optica spectrum disorders (NMOSD) and Myelin Oligodendrocyte Glycoprotein antibody disease (MOGAD, aka Anti-MOG associated encephalomyelitis) are CNS inflammatory demyelinating diseases whose primary pathology reflect deposition of IgG antibodies and activated complement. The great majority of NMOSD patients show IgG specificity for aquaporin-4 (AQP4), with consequent primary astrocytopathy and secondary demyelination. MOG specificity underlies primary oligodendrocyte pathology in MOGAD. Objectives: To examine pathomechanism for NMOSD and MOGAD using mouse models for disease pathology. Methods: Experimental NMOSD was induced by injection of patient-derived purified IgG, with human complement, either by stereotactic intrastriatal injection or by

intrathecal injection to cerebrospinal fluid. For MOGAD, a monoclonal MOG-specific IgG2a was stereotactically injected with mouse complement to the corpus callosum. Results: Intrathecal AQP4-IgG+complement induced subpial and periventricular NMOSD-like lesions and blood-brain barrier breakdown in brain and optic nerve. Blood-brain barrier breakdown co-localized with sites of human IgG and complement deposition. Striatal pathology included loss of AQP4 and GFAP staining, indicating primary astrocytopathy, with local microgliosis and deposition of activated complement. Anti-MOG+complement induced demyelination in corpus callosum, with deposition of activated complement. Control IgGs did not induce pathology. Neither NMOSD nor MOGAD pathology could be induced in mice lacking the Type I interferon (IFNI) receptor IFNAR. Microglia in striatum showed a strong IFNI transcriptomic footprint and NMOSD pathology was exacerbated by IFNβ. Depletion of microglia led to suppression of pathology and decrease of IFNI signature genes. Conclusion: NMOSD and MOGAD pathology in animal models has been demonstrated by IgG transfer, dependent on IFNI response with a key role for microglia.

Keywords: NMOSD – MOGAD – antibody – Interferon – microglia

W9.5

CD20+ T cells – a T cell disguised as a B cell

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Anti-CD20 antibody therapy is used to treat various inflammatory diseases including multiple sclerosis. B cells express high levels of CD20 and the strong efficacy of anti-CD20 antibody therapies in reducing disease progression has therefore been attributed to the depletion of B cells. With the discovery of a T cell subtype expressing low levels of CD20 that is also depleted by the anti-CD20 antibody therapies, great interest in the properties of CD20+ T cells has emerged. In our study we investigated a possible implication of CD20+ T cells in the pathogenesis of multiple sclerosis. This showed that CD20+ T cells are proinflammatory cells with a high proliferative capacity to CNS antigens and a great CNS-migration potential. In coherence, we found that CD20+ T cells were enriched in the CSF of patients with multiple sclerosis and that the prevalence of CSF-resident CD20+ T cells correlated positively with disease severity. These data indicate a role of CD20+ T cells in the pathogenesis of MS and suggest that depletion of CD20+ T cells contributes to the positive treatment effect of anti-CD20 antibody therapies.

Keywords: Anti-CD20 antibody therapy – CD20+ T cells – multiple sclerosis

clinical neuropathology
Abstracts

ISSN 0722-5091

DOI 10.5414/NPP40M01
epub: May 20, 2021

12th European Congress of Neuropathology

Virtual, May 31st – June 3rd, 2021

Organized by the Scandinavian Neuropathological Society (SNS) in conjunction with the European Confederation of Neuropathological Societies (Euro-CNS)
Congress President and Chair Scientific Program Committee: Bjarne Winther Kristensen, Copenhagen, Denmark

Regular (Poster) Abstracts

Vascular dementia

P001

Cortical raspberries – a sign of compromised perfusion?

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Introduction: We recently described a microvascular structure in the cerebral cortex. These structures, termed "raspberries" due to their appearance in developed forms, are minimal arteriolar structures with a diameter of 10 – 80 micrometers and with 3 – 15 or more vascular lumen. They hypothetically represent a reactive angiogenetic change to a noxious stimulus, possibly recurrent hypoperfusion. Hypothetically, several cardiovascular risk factors and deleterious hemodynamic events may interplay. Methods: We compared the frequency of raspberries in 10 neuropathologically examined deceased individuals with vascular dementia (VaD) and in 9 non-demented control cases (CC). In all 10 VaD cases and in 4 CC, concurrent cardiovascular disease was noted. In each case, a 20-mm length of a hematoxylin-eosin stained section from the anterior frontal, temporal and parietal and/or occipital cortex was examined for quantitation of raspberries. Results: In VaD, the group mean raspberry count was 3.8 (1.3 – 4.8 raspberries/cm cortex), while in CC the mean count was 0.6 (0.0 – 1.8 raspberries/cm) ($p < 0.001$). The VaD group had numerous raspberries with 4 lumen and several raspberries built of 10 – 15 lumen, whereas 3-lumen raspberries predominated among CC. Conclusion: Our findings lend support for the theory that cortical raspberries are formed through angiogenesis due to recurrent hypoperfusion of brains subject to widespread vascular pathology. Next, we aim to compare the prevalence of cardiovascular risk factors and severe lowering of blood pressure between individuals with high and low raspberry frequencies.

Keywords: Brain ischemia – neovascularization – vascular dementia.

*The regular abstracts will be presented as ePosters. In addition, each ePoster will be presented orally in one of the following ways: as a short presentation in a Symposium (SY) or Workshop (WS), as a short Free Communication, or as a short oral Quick Pitch in the Quick Pitch sessions. For the timetable with all presentation details, please visit our Congress website: www.ecnp2021.dk

P002

The effect of atherosclerosis in the neurovascular unit

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Introduction: Neuroinflammation is a prominent feature of the Apolipoprotein E knockout ($ApoE^{-/-}$) mouse model of atherosclerosis. Methods: The current study tested the hypothesis that microvascular damage related to systemic atherosclerosis induces cell specific changes which lead to neurovascular unit (NVU) dysfunction, contributing to neuroinflammation and neurodegeneration. Detailed immunohistological assessment of astrocytes (GFAP), microglia (IBA-1) and endothelial cells (ICAM-1) was performed on $ApoE^{-/-}$mice fed on a high fat diet (HFD) (n = 6 – 12) and on a low fat diet (n = 7 – 13). The hippocampus was isolated using laser capture microdissection and the transcriptomic profile characterized using microarray analysis. Results: $GFAP^+$ astrocytes ($p = 0.03$) and $ICAM1^+$ endothelial cells ($p = 0.01$) were significantly higher in the corpus collosum of animals fed on a HFD. Significantly higher levels of $Iba\text{-}1^+$ microglia were detected in the hippocampus ($p = 0.002$), corpus callosum ($p = 0.004$), and cerebral cortex ($p = 0.004$). Transcriptomic analysis of the hippocampus indicated significant downregulation of the endoplasmic reticulum associated degradation pathway ($p = 0.003$) and calcium signaling ($p = 0.002$); and a significant upregulation of metabolic ($p < 0.001$) and inflammatory pathways ($p = 0.006$) in animals fed on a HFD. Conclusion: These findings indicate that systemic atherosclerosis is associated with changes in the cerebral microvasculature, affecting astrocyte, microglial and endothelial cell responses. The gene expression changes associated with HFD and inflammation suggest that systemic atherosclerosis may be associated with cellular pathway alterations in the NVU. Future work is required to establish the importance of this in producing alterations in the brain.

Keywords: Atherosclerosis – ApoE – vascular dementia

FTD and ALS

P003

Pathological background of aphasia associated with neurodegenerative diseases

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Background: Pathological background of aphasia has been reported to be heterogenous. Purpose: In order to confirm the pathological background of aphasia in neurodegenerative disorders. Methods: Pathological findings were retrospectively reviewed in patients who had aphasia in medical records among 6200 autopsied brains of our brain resource center. Results: Fifteen patients were found among 6,200 autopsied brains, including 6 semantic, 2 transcortical sensory, 6 nonfluent/agrammatic, and 1 logopenic aphasia. Neuropathology of 6 patients with semantic aphasia were 3 frontotemporal lobar degeneration-motor neuron disease (FTLD-MND) with TDP type B, 1 Pick disease, and 2 FTLD-TDP associated with Alzheimer disease. Semantic aphasia showed atrophy in anterior temporal lobes including temporal pole, predominantly in the left side. Two patients with transcortical sensory aphasia were FTLD-MND-TDP. The distribution showed anterior frontotemporal lobes. Six patients with nonfluent/agrammatic aphasia were 1 corticobasal degeneration, 3 progressive supranuclear palsy, 1 Pick disease, and 1 FTLD-TDP. Nonfluent/agrammatic aphasia presented atrophy in posterior frontal, insular, superior temporal, and precentral regions. One patient with logopenic aphasia was dementia with Lewy bodies (diffuse neocortical type) associated with Alzheimer disease. Conclusion: Clinical phenotypes highly correlated with severity and distribution of cortical neuronal degeneration. Tauopathy and TDP-43 proteinopathy are the most vulnerable molecules in progressive aphasia syndromes.

Keywords: Aphasia – neurodegenerative disorder – tau – TDP-43

P004

Neuropathologic diversity in four tauopathy cases with duplication of the *MAPT* gene

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Introduction: Microduplication of the *MAPT* gene was initially described in patients with episodic memory impairment that clinically mimicked Alzheimer disease. Recently, two subjects harboring the same duplication

have been reported presenting an extrapyramidal syndrome and gait disorder, suggesting phenotypic variability. Objectives: We have characterized histologically four cases harboring a *MAPT* microduplication, including the two recently described subjects with extrapyramidal syndrome. Cases: The cases included three males and one female with ages at onset ranging from 37 to 57 years (mean age, 48.5 years). Two of the cases presented with memory impairment and in the other two cases an akinetic-rigid syndrome predominated at clinical examination. Histological assessment with H-E, tau immunohistochemistry including the phospho-tau antibody (pSer202/Thr205) AT8, phospho-tau (Thr212/Ser214) antibody AT100, the 3- and 4-repeat specific tau antibodies as well as silver histochemical stainings were done in all cases. Results: Neuropathologic examination showed a tauopathy with immunoreactive lesions that ranged from a 3R/4R tauopathy with a cortico-basal distribution to a 4R tauopathy with a distribution localized to the brainstem and basal ganglia. Conclusion: *MAPT* duplication can lead to a primary tauopathy with variable phenotypic spectrum with tau pathology ranging from 3R to 4R-tau predominant aggregates.

Keywords: Tau – MAPT – MAPT duplication – tauopathy

P005

FTLD-UPS in CHMP2B-FTD

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Introduction: Frontotemporal dementia linked to chromosome 3 (FTD-3) is a rare form of frontotemporal dementia caused by mutations in CHMP2B. The disease has been reported in a large Danish family and in a single Belgian patient, with different mutations identified, leading to C-terminal truncations of CHMP2B of 36 and 49 amino acids respectively. Symptoms include onset of dementia in the late 50s with changes in behavior and personality (disinhibition, apathy, ritualized behavior, loss of emotion, changed eating habits, dyscalculia, and dynamic aphasia leading to mutism). In some patients, motor symptoms develop after +4 years (asymmetric akinetic rigid syndrome with arm and gait dystonia). Objectives: The neuropathological findings have previously been described as FTLD-UPS based on autopsies in 5 patients in the Danish family. We now present data from 13 Danish patients and importantly, the patient from the Belgian family with a distinct CHMP2B mutation. Results: Immunohistochemical staining for ubiquitin, and p62, revealed varying numbers of round, cytoplasmic inclusions in the granule cells of the hippocampal dentate area and in cortical neurons in widespread cortical areas, as well as occasional inclusions in neurons in brainstem nuclei and motor neurons in the spinal cord. Staining for TDP-43, pTDP-43 and FUS were negative. Staining for tauprotein only revealed a few tangles in the transentorhinal cortex consistent with PART.

Keywords: FTLD-UPS – CHMP2B

P006

Frontotemporal lobar degeneration: neuropathological classification of cases from the Brain Collection at Aarhus University Hospital, Denmark

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Introduction: The brain collection at Aarhus University Hospital (Risskov), Denmark, includes more than 9,000 whole brains collected and studied in the years 1945 – 1982. 5,500 were diagnosed with dementia. Approximately one half of these patients were clinically diagnosed with Alzheimer's disease (AD), a smaller percentage was diagnosed with Pick's disease, and the rest had vascular dementia. The primary neuropathological diagnosis was performed before the introduction of immunohistochemistry and was largely based on silver-stained tissue sections allowing that enabled visualization of plaques and tangles characteristic of AD and Pick bodies in Pick's disease, which was used as a common clinical term for all non-AD dementia (frontotemporal dementia (FTD)) forms. Today, Pick's disease is one of many disease entities in the large group of diseases characterized by frontotemporal lobar degeneration (FTLD) which is the neuropathologic term for changes in brains of patients with FTD. Methods: We browsed the clinical journals at Risskov and identified 67 patients with a clinical diagnosis of frontotemporal dementia (FTD) or Pick's disease. These cases were reclassified using modern immunohistochemistry on sections from paraffin-embedded tissue blocks from these patients. We stained the sections with antibodies against tau (AT8, RD3, and RD4), p62, TDP-43, pTDP-43, FUS, asynuclein and Ab1-42. Results: The revised neuropathological diagnosis fell into the follow-

ing categories: 13 patients with AD, 25 patients with FTLD-tau (22 Pick's disease, 2 corticobasal degeneration, 1 progressive supranuclear palsy), 26 patients with FTLD-TDP, 4 patients with FTLD-UPS (3 of which belong to the Danish family with CHMP2B mutation).

Keywords: FTLD – brain bank

P007

Atypical frontotemporal lobar degeneration with bizarre glia, 4 > 3 repeat astrogliopathy and distinct TDP43 inclusions. Report of two cases

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Background: Rare familial cases of frontotemporal dementia (FTD) have shown an infrequent astrocyte predominant 4 > 3R tauopathy. Objectives: Describe two cases of FTD with these histological features including TDP43 co-expression. Methods: Neuropathological evaluation with immunohistochemical and ancillary studies. Results: Patient 1 died at 58 with a frontotemporal dementia with probable familial context. Patient 2 died at 72 with clinical diagnosis of posterior cortical atrophy with leukoencephalopathy. Both were female. Histology revealed a pattern of FTLD with frequent neurofibrillary tangles (NFT) and atypical pleomorphic glial cells with enlarged irregular nuclei and broad cytoplasm, particularly in case 2. Immunohistochemistry showed extensive astroglial predominant 4 > 3R tau pathology. Atypical astroglial inclusions in gray matter resembled mainly tufted or thorn shaped astrocytes, and very rarely astrocytic-plaque-like structures. Inclusions vere frequent in the glia limitans of the perivascular, subpial and subependymal regions and throughout the grey and white matter. They were abundant in cortical areas, limbic system, basal ganglia, brain stem and striking in the cerebellar cortex projecting from Bergmann glia to the molecular layer. NFTs were immunoreactive for 3R+4R tau with predominance in subcortical areas. pTDP43 accumulated in atypical astroglial cells and in neurons, mainly as globular or diffuse/dash like inclusions, spanning neocortex, basal nuclei, limbic system, brainstem and cerebellum, including Bergmann glia. No MAPT mutations or duplications were observed. Conclusion: We present two cases of astroglial predominant FTLD-tau presenting as familial FTD and posterior cortical atrophy, respectively, with atypical glial pathology and unique 4 > 3R tau and pTDP43 patterns.

Keywords: Astroglial tauopathy – glia limitans – pTDP43 – TDP43 – FTLD-Tau

P008

A de novo p.S320F (c959C > T) microtubule-associated protein tau gene mutation causes a Pick´s disease-like pathology with a predominant 3-repeat-tau component

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Introduction: MAPT mutations can cause fronto-temporal lobar degeneration associated with 3repeat (3R) and/or 4R tauopathies, depending on the location of each mutation. Here, we report a new subject with a de novo p.S320F (c959C > T) MAPT mutation showing Pick-like neuropathology, confirming findings of a previous description. Methods: Review of clinical history, ancillary tests and detailed neuropathological work-up. Results: A 42-year-old man developed progressive cognitive decline with difficulty in naming, words comprehension and mild-behavioral impairment. Neuropsychological examination revealed aphasia with semantic memory impairment, severe anomia and moderate executive dysfunction. MRI demonstrated severe bilateral (left-predominant) temporal atrophy. The clinical diagnosis was a semantic variant of primary progressive aphasia. Symptoms worsened during follow-up and mild parkinsonism appeared at age 52. He died 12 years after symptom onset. Genetic analysis demonstrated a p.S320F (c959C > T) MAPT mutation in the proband but not in his parents. Neuropathology showed a fronto-temporal lobar degeneration with prominent neuronal loss and gliosis, particularly involving superficial cortical layers and the limbic system. Extensive tau-pathology affected cortical and subcortical areas and was dominated by threads and variegate neuronal (irregular tangles, pretangles and frequent spherical inclusions, particularly in superficial layers and the den-

tate fascia) as well as tiny spherical oligodendroglial inclusions in white matter. This pathology was predominantly, but not exclusively composed of 3R-tau isoforms and was suggestive of Pick-type pathology with a mild 4R-tau component. Conclusion: Pick's disease-like pathology can be caused by de novo MAPT mutations. Genetic screening could be therefore useful in selected sporadic cases.

Keywords: MAPT mutation – Pick's disease-like pathology

P009 /SY 7.3

Cognitive decline in amyotrophic lateral sclerosis: neuropathological substrate and genetic determinants

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Introduction: Cognitive impairment and behavioral changes in amyotrophic lateral sclerosis (ALS) are now recognized as part of the disease. Whether it is solely related to the extent of TDP-43 pathology is currently unclear. Purpose: We aim to evaluate the influence of age, genetics, neuropathological features and concomitant pathologies on cognitive impairment in ALS patients. Methods: We analyzed a postmortem series of 104 ALS patients and retrospectively reviewed clinical and neuropathological data. We assessed the burden and extent of concomitant pathologies, the role of *APOE* ε4 and mutations, and correlated these findings with cognitive status. We performed a logistic regression model to identify which pathologies are related to cognitive impairment. Results: Cognitive decline was recorded in 38.5% of the subjects. Neuropathological features of frontotemporal lobar degeneration (FTLD) were found in 32.7%, explaining most, but not all, cases with cognitive impairment. Extent of TDP-43 pathology and the presence of hippocampal sclerosis were associated with cognitive impairment. Mutation carriers presented a higher burden of TDP-43 pathology and FTLD more frequently than sporadic cases. Most cases (89.4%) presented some degree of concomitant pathologies. The presence of concomitant pathologies was associated with older age at death. FTLD, but also Alzheimer's disease, were the predominant underlying pathologies explaining the cognitive impairment in ALS patients. Conclusion: FTLD explained the presence of cognitive decline in most but not all ALS cases, while other non-FTLD related findings can influence the cognitive status, particularly in older age groups.

Keywords: Amyotrophic lateral sclerosis – frontotemporal dementia – frontotemporal lobar degeneration – TDP-43

P010

Frontotemporal lobar degeneration due to a novel *GRN* nonsense mutation with an associated 4-repeat tauopathy

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Introduction: Secondary tauopathies, as found in association to an increasing number of neurodegenerative pathologies, may contain some clues for the pathogenesis of the anomalous tau protein, and for the phenotypic diversity of tauopathies. We recently described a family with autosomal dominant frontotemporal lobar degeneration (FTLD) due to a novel *GRN* nonsense mutation (c.5G > A: p.Trp2*). The proband's brain displayed a FTLD-TDP with a characteristic type-A pattern, and an extense glial tau(+) inclusions consistent with aging-related tau astrogliopathy (ARTAG). Objectives: Here we describe the postmortem neuropathological findings of a second affected member of the family, a niece of the proband. Methods: A full neuropathological examination of the donated brain was performed at the CIEN Tissue Bank. The patient was a female, 56 years of age at death, that initially displayed apathy and decreased verbal fluency, and two years later aphasia with a prominent dysexecutive syndrome. Immunohistochemical stains included a battery of antibodies for TDP-43(+) and tau(+) pathologies. Results: Macroscopic examination disclosed severe global atrophy of the cerebral hemispheres with prominent frontotemporal atrophy. Histological findings were consistent with type A DLFT-TDP, with extense cortical and subcortical TDP-43(+) inclusions. Additionally, frequent tau(+) inclusions were observed in the anterior medial temporal lobe (pretangles and tangles) and subcortical nuclei (granular tau+ astrocytes), with a staining pattern of a

4-repeat tauopathy. Conclusion: The study of a second affected member of the family confirms that this novel *GRN* mutation induces a 4-repeat tauopathy with a variable phenotype, combining in this case ARTAG-like and argyrophilic grain disease-like features.

Keywords: Frontotemporal dementia – secondary tauopathies – GRN mutations – ARTAG

P011 /SY 7.4

Neuroanatomy of FTD: whole-brain correlations between symptoms and pathologies

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Background: Distinct pathologies accumulate in the brain and shape the heterogeneous clinical presentation of frontotemporal dementia (FTD). It is unknown how regional pathological burden links to symptoms and what the role of co-occurring pathologies is. Objectives: To investigate how early FTD symptoms correlate to the burden of multiple pathologies throughout the brain. Methods: Post-mortem brain tissue of 88 frontotemporal lobar degeneration (FTLD) donors was dissected into twenty standard brain regions (BR) and stained for phosphorylated TAR DNA-binding protein 43 (pTDP-43), phosphorylated tau, fused-in-sarcoma (FUS), amyloid-beta (Aβ), and alpha-synuclein. The burden of each pathological protein in each BR was quantified. Psychiatric, behavioral, language, and motor symptoms in the first three years from disease onset were assessed. Whole-brain clinico-pathological partial correlations were calculated. Results: Positive partial correlations ($p < 0.01$) were found between hippocampal TDP-43 and hallucinations ($R = 0.23$), perseverative-compulsive behavior ($R = 0.25$), depression ($R = 0.28$), and mania ($R = 0.32$). Tau in the substantia nigra and locus coeruleus was linked to depression ($R = 0.25$, $R = 0.24$). Both TDP-43 and Aβ in the subthalamus were associated with disinhibition ($R = 0.23$, $R = 0.25$), while apathy correlated with both TDP-43 and tau in the parietal lobe ($R = 0.27$, $R = 0.24$). Conclusion: Neuropsychiatric symptoms of FTD are linked to pathology burden in BR beyond the frontal lobes, including subcortical structures such as the hippocampus, the substantia nigra and locus coeruleus. Co-occurring pathologies are not simple bystanders, but could play a role in configuring FTD clinical phenotype.

Keywords: FTD – FTLD – clinico-pathological correlations – neuropsychiatric – subcortical

P012

Using an induced pluripotent stem cell model of frontotemporal dementia to identify altered metabolic profiles in patient-derived neurons

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Introduction: Frontotemporal dementia (FTD) is a heterogeneous group of early-onset dementias leading to impairment of behavior, language and cognition. FTD can be caused by mutations in the gene *MAPT* encoding the microtubule-associated protein TAU leading to pronounced atrophy of the frontal and temporal lobes, basal ganglia and brain stem areas. Because of limited availability of neural cells from patients' brains, the underlying mechanisms of neurodegeneration in FTD are poorly understood. Methods: Here, we differentiated induced pluripotent stem cells (iPSCs) from individuals with the FTD-associated N279K mutation in *MAPT* as well as control (Ctrl) cells without *MAPT* mutation into mature neurons. Conclusion: Patient iPSC-derived neurons demonstrated TAU pathology, an impairment of neurite outgrowth and an increased but reversible oxidative stress response to inhibition of mitochondrial respiration. FTD neurons also showed altered metabolic profiles with an increased basal mitochondrial respiration, increased ATP production and an increased maximal respiratory capacity as measured by neuronal oxygen consumption indicating an increased energy demand in these cells. Interestingly, these changes could be rescued by repairing the *MAPT* mutation in FTD cells using CRISPR/CAS9 technology to generate isogenic gene-corrected Ctrl cells. Metabolomics mass spectrometry on FTD and Ctrl neurons indicated alterations in the intermediary metabolism in FTD neurons with an increased usage of glucose and glutamine as energy fuels. These findings demonstrate that FTD patient iPSC-derived neurons comprise a powerful tool to identify disease phenotypes in these cells at risk, which could be used as a cellular platform for high-throughput drug screening assays to identify potential therapeutic targets in FTD.

Keywords: FTD – frontotemporal dementia – MAPT – iPSC – metabolic

Prion diseases

P013

Regional differences in neuroinflammation-associated gene expression in the brain of sporadic Creutzfeldt-Jakob disease patients

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Introduction: Neuroinflammation is an essential part of neurodegeneration. Yet, the current understanding of neuroinflammation-associated molecular events in distinct brain regions of prion disease patients is insufficient to lay the ground for effective treatment strategies targeting this complex neuropathological process. Methods: To address this problem, we analyzed the expression of 800 neuroinflammation-associated genes to create a profile of biological processes taking place in the frontal cortex and cerebellum of patients who suffered from sporadic Creutzfeldt–Jakob disease. The analysis was performed using NanoString nCounter technology with human neuroinflammation panel+. Conclusion: The observed gene expression patterns were regionally and sub-regionally distinct, suggesting a variable neuroinflammatory response. Interestingly, the observed differences could not be explained by the molecular subtypes of sporadic Creutzfeldt-Jakob disease. Furthermore, analyses of canonical pathways and upstream regulators based on differentially expressed genes indicated an overlap between biological processes taking place in different brain regions. This suggests that even smaller-scale spatial data reflecting subtle changes in brain cells' functional heterogeneity and their immediate pathologic microenvironments are needed to explain the observed differential gene expression in a greater detail.

Keywords: Prion disease – neuroinflammation – regional brain microenvironment – gene expression analysis

P014

Molecular characterization of the Danish prion diseases cohort with special emphasis on rare and unique Cases

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Introduction: Human prion diseases are phenotypically heterogeneous neurodegenerative diseases caused by host-encoded proteins that are capable of acquisition of an alternative conformation and self-propagation. Methods: The purpose of this study was to perform an updated reclassification of all definite prion disease cases with available fresh-frozen samples referred to the Danish Reference Center over the past 40 years, putting a special emphasis on the molecular characterization of novel disease subtypes. Conclusion: Investigation of the Danish prion diseases cohort revealed rare sporadic Creutzfeldt-Jakob disease cases with mixed subtypes and subtypes with previously uncharacterized white matter plaques, a new case of sporadic fatal insomnia, and 3 novel mutations, including 2 large octapeptide repeat insertions, and a point mutation in the prion protein gene. The evaluation of methionine and valine distribution at codon 129 among the prion disease patients in the cohort revealed the increased prevalence of methionine homozygotes compared to the general population. This observation was in line with the prevalence reported in other Caucasian prion disease cohort studies. Reclassification of the old prion diseases cohort revealed unique cases, the molecular characterization of which improves prion diseases classification, diagnostic accuracy, genetic counseling of affected families, and the understanding of disease biology.

Keywords: Prion disease – classification – molecular characterization

P015

Contribution of neuropathology for the diagnosis and surveillance of prion diseases in Brazil – case series from 2005 – 2020

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Short Background: Prion diseases are rapidly progressive and fatal neurodegenerative diseases caused by anomalous cerebral accumulation of prion protein (PrP^{Sc}). They include Creutzfeldt-Jakob disease (CJD) and its subtypes: sporadic (sCJD), hereditary (hCJD), iatrogenic (iaCJD) and variant (vCJD); Gerstmann-Sträussler-Scheinker syndrome (GSS); fatal familial insomnia (FFI); and kuru. A definitive diagnosis requires neuropathological analysis, with immunoreactivity for

PrP^{Sc}. Since 2005 we are the National reference center for the pathological diagnosis and surveillance of prion diseases in Brazil. Objectives: To verify how many of the suspected cases were immunohistochemically confirmed during a 16-year-period and to describe the morphological and immunohistochemical patterns of the confirmed cases. Methods: Ninety-nine cases were received from all regions of Brazil from 2005 to 2020. Histological sections were stained with hematoxylin and eosin for morphological analysis and immunohistochemical studies for PrP^{Sc} detection were performed. Results: Eighty-five/99 cases were positive for PrP^{Sc}. There were 79 sCJD, 2 hCJD, 2 GSS, and 2 iaCJD. No cases of FFI, kuru, or vCJD were identified in this series. The low national frequency was probably not accurate because adhesion to the project was not homogeneous in all states. All positive cases for PrP^{Sc} showed cortical spongiosis, neuronal loss and gliosis. The main immunostaining patterns were synaptic and perivacuolar, but multicentric plaques were also observed in the GSS cases. Conclusion: Most cases with immunohistochemical confirmation in Brazil are sCJD, but there are also hereditary disorders (hCJD and GSS) and iaCJD. The absence of vCDJ is of great importance for a Country's health and economy.

Keywords: Prion diseases – immunodetection – surveillance

Dementia – Alzheimer

P016

Biochemical variants of beta-amyloid and their association with hyperphosphorylated tau in brain biopsies from subjects with idiopathic normal pressure hydrocephalus

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Background: Idiopathic Normal Pressure Hydrocephalus (iNPH) is a disease of elderly causing cognitive-, gait- and urinary impairment that can be reversed by a ventriculoperitoneal shunt. In a few centres, a brain biopsy is taken during the shunt insertion and Alzheimer's disease neuropathological change (ADNC) has been detected in a substantial number of these biopsies. Objectives: To study different variants of beta-amyloid (Aβ), and their association with hyperphosphorylated tau (HPτ), in iNPH subjects and in a tissue microarray (TMA) including post-mortem brain tissue from subjects with different stages of ADNC. Methods: 127 iNPH brain biopsies with notable Aβ pathology, and a TMA with samples from 19 subjects with different ADNC stages, were assessed and immunohistochemically (IHC) stained with antibodies towards different Aβ variants and HPτ. The outcome was assessed semi-quantitatively. Results: All iNPH biopsies displayed Aβ and 91% HPτ. All markers increased with age and the extent of pyroglutamylated (py) Aβ was higher than phosphorylated (p) Aβ. In the whole cohort all Aβ markers correlated with each other and with HPτ. In subjects ≥ 75 years, the HPτ correlated only with the pyAβ and pAβ. In the TMA all the markers increased with the level of ADNC and pyAβ was higher than pAβ. Conclusion: In iNPH the extent of pyAβ and pAβ increase with age and the pyAβ precedes formation of pAβ. The modified Aβ variants are associated with the extent of HPτ pathology. Our findings in iNPH mirrors what previously described in AD, suggesting that iNPH subjects also suffer from AD.

Keywords: Idiopathic normal pressure hydrocephalus – Alzheimer's disease neuropathological change – biochemical beta-amyloid variants – hyperphosphorylated tau

P017/SY 12.4

Alzheimer's disease neuropathological change and loss of neuropil in patients with idiopathic normal pressure hydrocephalus, a model of Alzheimer's disease

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Background: Idiopathic normal pressure hydrocephalus (iNPH) is a neurological condition in elderly presenting with gait-, cognitive-, and urinary symptoms. Some centers in the world are taking a brain biopsy from iNPH subjects during a curative ventriculoperitoneal shunt (VPS) insertion. Substantial number of iNPH patients display Alzheimer's disease neuropathological change (ADNC) in their brain biopsies but no hallmark iNPH lesion is identified. ADNC in iNPH subjects is associated with worse shunt response and progress into dementia. Objectives: To assess ADNC and neuronal population in brain biopsies from iNPH patients. Methods: 95 brain biopsies from iNPH patients aged 75 – 79 years were assessed and stained with antibodies towards ADNC markers, neuronal markers and a subset of cases with synaptophysin (SYP). The stained sections were scanned and morphometrically analyzed as stained area fraction (SAF). Results: 63% of the iNPH patients displayed β-amyloid (Aβ) in their biopsies and 61% displayed hyperphosphorylated τ (HPτ). Concomitant ADNC was seen in 49% of subjects. Females were more affected than men. The ADNC markers correlated with each other and with age at a borderline level. The extent of NeuronalNuclei (NeuN) marker within a defined area increased with age. When comparing the association of NeuN and SYP, the SAF/SYP was lower in cases with higher SAF/NeuN. Conclusion: Significant number of iNPH patients display ADNC. The extent of pathology indicates early AD. Females are more affected than men. There is notable neuronal preservation and loss

of synapses in the iNPH brain biopsies. Altogether, iNPH is a reliable model of AD.

Keywords: Idiopathic normal pressure hydrocephalus – Alzheimer's disease neuropathological change – amyloid-beta – hyperphosphorylated tau

P018/WS 1.5

Tau related changes in post mortem retina in Alzheimer's disease and other tauopathies

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Introduction: There is increased interest in in vivo labeling of Alzheimer's disease (AD) pathology in the retina as a non-invasive diagnostic approach. Objectives: In this study we assessed the presence of different tau isoforms in post-mortem retinas of AD as well as other neurodegenerative diseases associated with or without tau pathology. Methods: Post-mortem eyes were collected through the Netherlands Brain Bank from donors with AD (n = 15), frontotemporal lobar degeneration (FTLD; n = 6), other tauopathies (n = 3), and alpha-synucleinopathies (n = 12), as well as non-neurodegenerative controls (n = 16). Cross-sections of the superior-temporal quadrants were immunostained for total tau (HT7), early tau phosphorylation (AT8, AT100, AT270), 3R and 4R tau isoforms (RD3/RD4), late tau phosphorylation (pS422). Results: Total tau (HT-7) was observed in non-demented control and neurodegenerative cases. The presence of 3- and 4-repeat isoforms of tau varied within the inner plexiform layer (IPL) and outer plexiform layer (OPL) with more 3-repeat tau in the IPL and more 4-repeat tau in the OPL. Only AD and other tauopathy cases showed positive immunoreactivity for AT8. Tau phosphorylated at Ser422 was negative in all groups. Conclusion: In controls and cases, high levels of tau are present in the retina, mainly in the plexiform layers. Overall, tau phosphorylated at Ser202/Thr205 differentiates tauopathies from other neurodegenerative diseases and non-demented controls. Depending on the epitope, phosphorylated tau is a potential retinal biomarker for AD and other tauopathies.

Keywords: Alzheimer's disease – retina – tau

P019/WS 1.4

Aggregates of RNA binding proteins and ER chaperones linked to exosomes in granulovacuolar degeneration of the Alzheimer's disease brain

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Introduction: Granulovacuolar degeneration (GVD) occurs in Alzheimer's disease brain due to compromised autophagy. Endoplasmic reticulum function and RNA binding protein (RBP) homeostasis regulate autophagy. Objectives: The study was to define molecular mechanism (s) of GVD in AD focusing on regulation of ER Chaperones in GVD affected neurons, RBP homeostasis as a possible trigger for GVBs formation. Methods: A cohort of AD autopsy brains complemented by pR5 and APP/PS1 transgenic mice were examined using immunohistochemistry, immunofluorescence and western bloting. Results: We observed that the ER chaperones, GRP78, Sigma receptor 1 (SigR1), and VAPB were elevated in many AD patients' subicular neurons. However, those neurons which were affected by GVD showed lower chaperone levels. Consistent with this notion, granular, incipient pTau aggregates in human AD and pR5 tau transgenic mouse neurons were regularly co-localized with increased chaperone immunoreactivity, whereas neurons with mature neurofibrillary tangles lacked both the chaperone buildup. On the other hand, APP/PS1 transgenic mouse hippocampal neurons displayed only few GVBs-like vesicles. Identifying a potential trigger for GVD, we found cytoplasmic accumulations of RBPs including Matrin 3 and FUS as well as stress granules in GVBs of AD patients and pR5 mouse neurons. Interestingly, we observed that GVBs containing aggregated pTau and pTDP-43 were consistently co-localized with the exosomal marker Flotillin 1 in both AD and pR5 mice. Conclusion: We conclude that altered chaperone-mediated ER protein homeostasis and impaired autophagy manifesting in GVD are linked to both pTau and RBP accumulation and that some GVBs might be targeted to exocytosis.

Keywords: Endoplasmic reticulum chaperones – Sigma receptor 1

(SigR1) – RNA binding proteins (RBP) – exosomes – granulovacuolar degeneration (GVD)

P020/SY 12.5

The coarse-grained plaque: a divergent Aβ plaque-type in early-onset Alzheimer's disease

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Introduction: Alzheimer's disease (AD) is characterized by amyloid-beta (Aβ) deposits. Previously, we observed an atypical Aβ deposit, referred to as the coarse-grained plaque. In this study, we evaluate the plaque's association with clinical disease and perform in-depth characterization. Methods: The plaque was semi-quantitatively scored in Aβ (6F3D) immunostaining of the middle frontal gyrus of Aβ-positive cases (n = 74), including non-demented (n = 15), early-onset (EO)AD (n = 38), and late-onset (LO)AD cases (n = 21). Brain regional distribution was scored in a subset of cases. In-depth characterization was done by studying the plaque's neuritic component, Aβ isoform composition, its neuroinflammatory component, and its vascular attribution using 3D confocal laser scanning microscopy. The plaque was compared to the classic cored plaque, cotton wool plaque, and CAA. Results: The coarse-grained plaque, a relatively large deposit, characterized as having multiple cores and Aβ-devoid pores, was prominent in the neocortex. The plaque was only observed in cases with clinical dementia and more frequently present in EOAD compared to LOAD. This plaque was associated with a homozygous APOE ε4 status and CAA. Similar to CAA but different from classic cored plaques, the coarse-grained plaque was predominantly composed of Aβ40. The plaque was distinctly associated with both intense neuroinflammation and vascular markers. 3D-microscopy revealed a particular Aβ40 shell structure and a direct relation with vessels. Conclusion: Based on its morphological and biochemical characteristics, the coarse-grained plaque is a divergent Aβ plaque-type associated with EOAD. Differences in Aβ processing and aggregation, neuroinflammatory response, and vascular clearance may presumably underlie the difference between coarse-grained plaques and other Aβ deposits.

Keywords: Alzheimer's disease – amyloid-beta – plaque – neuropathology

P021/WS 7.4

Subregional severity of proteinopathies in the hippocampus of late onset Alzheimer's disease (AD) and dementia with Lewy Bodies (DLB) patients

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Introduction: The hippocampus, as a key area of memory processes, is particularly vulnerable to neurodegeneration in AD and DLB. Even if AD and DLB patients share memory decline, the atrophy of the hippocampus does not follow the same pattern with the CA1 subfield being more severely touched in AD. Methods: To further understand this differential hippocampus deterioration, we investigated the distribution of typical AD and DLB protein deposits in the hippocampal subfields CA1, CA3 and dentate gyrus in a collection of human post-mortem samples (between the uncus and corpus geniculatum laterale) in AD, DLB, and age-matched control samples. For this purpose, we have immunostained thick sections for amyloid-β (Aβ), phosphorylated forms of tau (Ptau) and a-synuclein (Psyn). We have combined 3D high-resolution confocal microscopy and image analysis tools to characterize the protein deposits in the three hippocampal subfields of 29 individuals. Conclusion: We found an overlap of the following typical inclusions: Aß plaques, neurofibrillary tangles, and Lewy bodies, in all conditions and subfields. Our control group was mostly showing extremely low levels of PTau but some healthy individuals showed prominent Aβ and Psyn inclusions. Our volumetric analysis

of protein deposits showed subfield- and condition-dependent patterns with AD CA1 being characterized by a higher level of PTau. Furthermore, we identified a strong correlation between PTau and PSyn in all three subfields with a partial co-distribution at the cellular level. This report shows a complex overlapping of neuropathological features between AD and DLB but with disease-specific exacerbation of their burden in certain subfields.

Keywords: Alzheimer's disease – dementia with Lewy Bodies – Hippocampus

P022/WS 7.6

Diabetes is associated with vascular dementia, not Alzheimer's disease or Lewy Body Dementia

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Background: Cardiovascular disease (CaVD) and diabetes mellitus type II (DM) are known risk factors for Vascular dementia (VaD), and have also been proposed as risk factors for other major neurocognitive disorders, including Alzheimer's disease (AD). Objective: We aimed to investigate the prevalence of CaVD, hypertension (HT) and DM in a neuropathologically confirmed cohort of major neurocognitive disorders. Method: In a total of 329 neuropathologically examined cases with VaD (n = 106), AD (n = 81), Mixed VaD-AD (MD, n = 81) and Lewy body dementia (LBD, n = 61), the prevalence of DM and HT was noted from the referral documents and medical records (1-3). Concurrent CaVD as found at autopsy, was recorded. Results: Obtainable data presented as %. In the order of LBD, AD, MD and VaD, the prevalence of HT was 36%, 37%, 44%, and 74%, respectively. The prevalence of DM was 8, 12, 190 and 31%. Presence of concurrent cardiovascular disease at autopsy; myocardial infarction, myocardial hypertrophy, coronary and aortic sclerosis and nephrosclerosis, followed a similar pattern of prevalence as hypertension and DM. Discussion and conclusion: DM was not highly prevalent in AD and LBD, being less prevalent than in the Swedish population over 65 years (15.6 %) (nat. statistics). DM and HT and markers for CaVD are most frequent in VaD among major neurocognitive disorders. The association between DM and neurocognitive disorders is explained by the link between DM and vascular disease, in turn causing VaD which may be misdiagnosed as AD or LBD when neuropathological verification is not obtained.

Keywords: Alzheimer's disease – vascular dementia – diabetes – cardiovascular disease

P023/WS 1.3

Deconvolving the individual contributions of comorbid tau neuropathologies using deep learning

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Introduction: Co-occurrence of neurodegenerative diseases becomes increasingly common with age and has the potential to confound both disease specific biomarkers and treatment. The study of co-existent disorders is hindered by the lack of disease specific immunostaining reagents and quantitative tools to measure individual disease burden by neuropathology. Objective: The objective of this study is to develop a computational approach to quantify disease burden in cases of comorbid tau neuropathology. Methods: We developed a deep learning-based approach to quantify disease contribution in immunostained tissue sections. We first trained a deep learning network to identify clinically derived pathological features associated with two neurodegenerative diseases characterized by tau pathology: Alzheimer's disease (AD) and progressive supranuclear palsy (PSP). We then applied a data-driven probabilistic approach to model disease pathology based on the spatial distribution of identified pathological features in tissue samples. Results: In pure disease samples, our deep learning model identified disease-specific tau aggregates with an average accuracy of 91%. Probabilistic modeling of identified aggregates successfully discovered two distinct disease signatures that correlated well to AD and PSP. Finally, application of this approach to tissue samples with mixed AD and PSP pathology could identify local regions that strongly corresponded to each disease signature. Conclusion: These results demonstrate the feasibility of this approach to quantify the individual contributions of comorbid tau neuropathologies to overall disease and provide a tool to study the correlation of this contribution to clinical data in future work. Further testing and validation are needed to extend this approach to additional disorders.

Keywords: Tau comorbidity deep learning

P024/SY 12.6 WITHDRAWN

Systemic infection exacerbates cerebral hypoperfusion and blood-brain-barrier breakdown in Alzheimer's disease and vascular dementia

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Introduction: Reduced cerebral blood flow and leakiness of the blood-brain-barrier (BBB) are early contributors to cognitive decline and disease progression in Alzheimer's disease (AD). In clinical studies, systemic infection accelerates cognitive decline in dementia. Given the known effects of infection on extracranial vascular function, we hypothesized that the deleterious influence of systemic infection in dementia results at least partly from impaired cerebral vascular function. Objectives: To investigate the impact of terminal systemic infection on markers of cerebrovascular function, brain cytokines and Aβ levels, at different stages of AD and in vascular dementia (VaD). Methods: We examined superior temporal cortex from AD (n = 75), VaD (n = 22) and control brains (n = 46) stratified according to the presence of terminal systemic infection and grouped into Braak tangle stages (BS) 0 – II, III – IV, and V – VI. Brain cytokines were measured using Mesoscale Discovery Multiplex Assays. Aβ and markers of cerebrovascular function were measured by ELISA. Results: Biochemical markers of cerebral hypoperfusion (reduced MAG:PLP1 and increased VEGF), BBB leakiness (increased fibrinogen), endothelial activation (increased ICAM-1, VCAM-1 and E-selectin) and the levels of multiple brain cytokines were raised by systemic infection in both AD (independently of Aβ42 level) and VaD. Inverse relationships between cerebral perfusion and IFN-γ, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-13 and TNF-α were lost in BSIII-IV and BSV-VI brains from donors without infection, and in all Braak stages from those with infection. Conclusion: Systemic infection and associated neuroinflammation cause cerebrovascular dysfunction, exacerbating cerebral hypoperfusion and BBB breakdown in early AD (independently of Aβ) and in VaD.

Keywords: Alzheimer's disease – systemic infection – neuroinflammation

P025

Primary age-related tauopathy (PART) in a Finnish population-based study of the oldest old (Vantaa 85+)

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Introduction: Primary age-related tauopathy (PART) is a relatively new term used to describe the presence of Alzheimer's disease (AD) -type neurofibrillary tangles (NFT) without or with minimal coexisting amyloid-β (Aβ) plaques. Objectives: There are only few studies that have investigated PART in a population-based setting. The aim of this study was to assess the prevalence, genetic background and features of cognitive decline in an unselected elderly population. Methods: The population-based Vantaa 85+ Study includes all inhabitants of the city of Vantaa who were ≥ 85 years in 1991 (n = 601). Neuropathological assessment was possible in 301 subjects. 60 PART cases were identified by using the criteria of Crary et al. 2014, and they were compared to the non-PART subjects (n = 241), and to AD subjects with severe AD-type neuropathologic changes (n = 100). Results: The prevalence of PART was 19.9 % (n = 60/301) and that of definite PART was 5.3 % (n = 16/301). The number of subjects with dementia was significantly lower in the PART group (48.3%) when compared to the non-PART group (68.9%) and the AD group (89.0%), and the PART subjects had significantly higher MMSE scores, higher age at onset of dementia, and shorter disease duration. *APOE* ε4 allele frequency was lower and *APOE* ε2 allele frequency was higher in the PART group compared to the other groups. Conclusion: PART is one of the most common neuropathologic findings in the brains of > 85 year-olds. It is important to separate PART from classical AD and other neurodegenerative disorders in both clinical and neuropathological studies.

Keywords: Neurodegenerative diseases – PART – neurofibrillary tangles – amyloid plaques – oldest old

P025.5 Persistent oxidative DNA damage alters the neuronal transcriptome and impacts cell cycle regulation and mitochondrial function

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Background: Increased oxidative stress and accumulation of oxidative DNA damage are hallmarks of brain aging and neurodegeneration, but the effects of these mechanisms in neurons are not well characterized. Objectives: To investigate the effect of persistent oxidative DNA damage in the neuronal transcriptome using the Lund human mesencephalic cells (LUHMES), an immortalized human neuronal cell line that can be differentiated to post-mitotic neurons. Methods: We developed a model of persistent oxidative DNA damage by exposing differentiated LUHMES to a sub-lethal concentration of hydrogen peroxide following a "double-stress" protocol. We tracked the formation of oxidative DNA damage foci over 96 hours using immunocytochemical detection of γH2AX. We then conducted a detailed characterization of the neuronal transcriptome of "double-stressed" LUHMES at the 96-hours timepoint using microarray and performed qPCR and functional validation to confirm our findings. Results: A significant percentage of cells in the "double-stress" model remained positive for DNA damage

foci even 96 hours after stress, suggesting persistent oxidative DNA damage. Transcriptomic analysis of "double-stressed" LUHMES detected significantly altered expression of genes involved in the anaphase-promoting (APC/C) cell cycle regulatory complex, the ATR-dependent DNA damage repair pathway and mitochondrial electron transport chain. qPCR validation confirmed dysregulation of cell cycle and mitochondrial genes, and functional validation further indicated hyperactivation of mitochondrial Complex I. Conclusion: Persistent oxidative DNA damage significantly impacts gene expression of LUHMES. These changes are linked to cell cycle regulatory pathways and to mitochondrial complex I hyperactivation, which could contribute to maintaining an oxidative environment and promote neuronal dysfunction.

Keywords: Neuron – DNA damage – cell cycle

P026/WS 7.5

Limbic-predominant age-related TDP-43 encephalopathy (LATE) in a cohort of patients with dementia: evidence of an early phase of hippocampal sclerosis

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Introduction: LATE, a recently described entity, encompasses previous pathological conditions as hippocampal sclerosis (HS) and TDP-43 proteinopathy associated with advanced age and/or Alzheimer's pathology. LATE includes patients either with or without HS (HS+/HS–), and develops along three stages of TDP-43 deposition in/or beyond the medial temporal lobe (MTL). Objectives: To characterize an early HS phase and associated neurodegenerative postmortem and premortem cognitive findings. Methods: A full postmortem neuropathological study was performed in 160 brains from a clinicopathological cohort of institutionalized dementia patients (Vallecas Alzheimer's Center) (age at death = 87.4 ± 6.6, sex ratio = 79.4% females) with a predominant primary diagnosis of Alzheimer's disease and a high prevalence of comorbid pathologies. An initial phase of HS (pre-HS) was defined based on morphologic criteria (distal CA1 sector). Results: 36.9% of patients displayed full HS, while 32.1% additional cases fulfilled criteria for early HS. A staging system of HS including pre-HS was built, which showed significant correlation with age at death, survival time, brain weight, MTL atrophy, Braak tau, α-synuclein and LATE staging, and cognitive antemortem variables. The HS+ group, including pre-HS, was significantly associated to female sex and amygdala-predominant Lewy body pathology. Conclusion: HS and LATE are highly prevalent in very aged dementia cohorts with a high burden of comorbidity and are strongly associated with survival time, brain atrophy and both Alzheimer and Lewy-body pathologies. An initial phase of HS can be identified in a substantial proportion of patients with LATE that may offer some pathogenic clues of the neurodegenerative process.

Keywords: Hippocampal sclerosis – LATE – TDP-43 pathology – dementia – Alzheimer's disease

P026Z

Lemur tyrosine kinase 2 (LMTK2) level inversely correlates with phospho-tau in neuropathological stages of Alzheimer's disease

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Background: Alzheimer's disease (AD) is the most common neurodegenerative dementia. Mapping the pathomechanism and providing novel therapeutic options have paramount significance. Introduction: Recent studies have proposed the role of LMTK2 in AD. However, its expression pattern and association with the pathognomonic neurofibrillary tangles (NFTs) in different brain regions and neuropathological stages of AD is not clear. Methods: We performed chromogenic (CHR) LMTK2 and fluorescent phospho-tau/LMTK2 double-labelling (FDL) immunohistochemistry (IHC) on 10-10 postmortem middle frontal gyrus (MFG) and anterior hippocampus (aHPC) samples with early and late neuropathological stages of AD. MFG in the early stage was our 'endogenous control' region as it is not affected by NFTs. Results: Semi-quantitative CHR-IHC intensity scoring revealed significantly higher ($p < 0.001$) LMTK2 values in this group compared to NFT-affected regions. FDL-IHC demonstrated LMTK2 predominance in the endogenous control region, while phospho-tau overburden and decreased LMTK2 immunolabeling were detected in NFT-affected groups (aHPC in early and both regions in late stage). Pearson's correlation coefficient showed strong negative correlation between phospho-tau/LMTK2 signals within each group. Conclusion: According to our results LMTK2 expression is inversely proportional to the extent of NFT pathology, as well as decreased LMTK2 level is not a general feature in AD brain, rather it is characteristic to the NFT-affected regions.

Keywords: Alzheimer's disease – immunohistochemistry – human

P026Z.1

Analysis of lemur tyrosine kinase-2 expression in neurodegenerative dementias

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Introduction: In physiological neurons harmonized axonal transport is essential for normal synaptic function. Changes in Lemur tyrosine kinase-2 (LMTK2) level may contribute to the disruption of molecular transport leading to synaptic loss and neurodegenerative processes. Purpose: Our aim was to characterize the LMTK2 expression in Alzheimer's disease (AD) and Dementia with Lewy bodies (DLB) and define the potential alterations compared to age-match controls. Methods: Formalin-fixed paraffin embedded tissues were collected from MRC London Neurodegenerative Diseases Brain Bank. The assessed brain region was determined by neuropathologist (TH), then we applied LMTK2 antibody and scanned the slides. Ten images/case were taken and post-processed with ImageJ software. We selected cells based on size, cytoplasmic volume and visibility of nuclei and identified the pyramidal cells as the target subgroup. We measured the mean grey value of these neurons and determined mean, median and mode intensity profiles for each case. Statistical data was calculated with SigmaStat software. Results: One-way ANOVA showed significant differences in mean and median intensity among the three (AD, DLB, control; $p < 0.001$) groups. Comparing two-two groups with T-test (AD-control, DLB-control; $p < 0.001$ and $p = 0.012$ respectively) and Mann-Whitney U test (AD-DLB; $p = 0.026$) there were statistically significant differences in mean and median intensities. Mode intensity values were significant only between AD and control groups with T-test ($p = 0.021$). Conclusion: Our results indicate significant changes in LTMK2 expression among age-match control and disease groups, as well as between AD and DLB patients. Further analysis of the protein's role in underlying pathomechanism may provide a promising new therapeutic target in dementias.

Keywords: Alzheimer's disease – Dementia with Lewy bodies

Movement disorders

P027

Multinodular vacuolating neuronal tumor arising in the setting of corticobasal degeneration shows relative exclusion of disease-associated tau immunoreactivity

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Introduction: Multinodular vacuolating neuronal tumor (MVNT) is a recently described neoplasm or hamartoma characterized by multinodular growth of an abnormal population of vacuolated neuronal cells, variably associated with epilepsy. Methods: We investigated an autopsy case of asymptomatic MVNT in a patient with 4R-tau proteinopathy corticobasal degeneration (CBD) using immunohistochemistry for tau antibodies targeting different phosphorylation, conformational, and isoform epitopes. Results: A 65-year-old man presented with gait apraxia, unilateral spasticity, and bradykinesia and deceased 8 years later. Incidentally, a multinodular lesion was discovered in the left frontal lobe on neuroimaging. At autopsy, the lesion extended from the periventricular white matter to the deep layers of the neocortex. Lesional cells showed neuronal morphology with vacuolation and expressed markers of both neuronal (synaptophysin) and glial (Olig2) lineage, consistent with MVNT. Neurodegenerative changes characteristic of CBD, including atrophy, neuronal loss, and gliosis of the frontotemporal lobes, ballooned neurons, and extensively distributed neuronal tau pathology, oligodendroglial coiled bodies, threads, and astrocytic plaques on tau immunostaining were seen. While the MVNT showed a similar epitope profile of tau-immunoreactivity (tau-ir) as the surrounding brain parenchyma, tumor nodules showed a marked relative reduction in tau-ir process density and absence of hallmark tau-ir lesions of CBD. Conclusion: We describe a rare coincidence of MVNT and CBD. Although MVNT nodules show a similar epitope profile of tau-ir, the decreased density may indicate that the distinct cellular composition of MVNT inhibits the generation/propagation of pathological tau into tumor nodules, with implications for the understanding of tau proteinopathy pathogenesis.

Keywords: Tauopathy – neurodegeneration – MVNT – tau – corticobasal degeneration

P028

Fulminant corticobasal degeneration mimicking autoimmune brainstem encephalitis

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Introduction: Rapidly progressive corticobasal degeneration (RP-CBD) was recently described as a distinct aggressive variant of CBD with characteristic neuropathological substrates resulting in a fulminant disease process clinically and pathologically. Objectives: To describe an autopsy case of RP-CBD. Methods: Clinical (video), neuroimage, neuropathological description. Results: A 51-year-old man presented with bilateral ptosis, vertical diplopia, and fatigue of six-month duration. The diagnostic workup for myasthenic syndromes was negative and in the following months, he developed progressive dysphagia, dysphonia, supranuclear ophthalmoplegia, cognitive decline, and asymmetric akinetic-rigid parkinsonism. Immunological testing revealed weakly positive anti-Ma2/Ta antibodies, negative on repeat testing. Brain MRI showed paramedian pons T2 hyperintensity and seven months later worsening with tegmental pontomesencephalic extension. Screening for occult neoplastic process and neuronal surface antibodies, including IgLON5, was negative. There was no response to immunomodulating therapies. Worsening bulbar dysfunction and cognitive decline led to severe deterioration. He died of bilateral pneumonia at age 54. The neuropathology study showed extensive neurodegenerative features (frontal cortex, basal ganglia, some thalamic nuclei and particularly severe in brainstem) with widespread cortico-subcortical 4-repeat tauopathy (neurofibrillary tangles, coiled bodies, extensive thread pathology and astrocytic plaques) extending to spinal cord, consistent with CBD in an advance disease stage. Conclusion: The severity of the brainstem pathology correlated with clinical presentation and neuroimaging findings. Remarkably, all previous RP-CBD reported cases were male. We highlight the atypical clinical presentation of this rare CBD form and the importance of identification of these rapid progressive variants, allowing to explore accurate biomarkers and genetic modifiers of these patients.

Keywords: Corticobasal degeneration – fulminant disease – brainstem

P029

Neuropathological investigation of lemur tyrosine kinase-2 (LMTK2) in Parkinson's disease dementia

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Introduction: Parkinson's disease is the most common neurodegenerative movement disorder frequently associated with dementia (PDD). Reduced lemur tyrosine kinase-2 (LMTK2) expression has been reported in Alzheimer's disease contributing to neurodegenerative processes. However, the role of LMTK2 in human PDD studies has not been investigated so far. Our aim was the fluorescent immunohistochemical (IF-IHC) characterization of LMTK2 in human PDD samples compared to age-matched controls. Methods: We selected 5-5 formalin-fixed paraffin embedded samples of PDD patients with severe neuropathological changes and age-matched controls. The investigated brain regions were middle frontal gyrus (MFG) and midbrain. Since, AD-type neurofibrillary tangles (NFT) may influence the analysis selection criteria for control samples were the absence of both α-synuclein (α-Syn) and NFT pathology. LMTK2 IF-IHC was performed according to the manufacturer's protocol. Then, we took 5 – 5 photos/cases at medium magnification. Digital image analysis was performed with ImageJ software. We determined the mean grayscale intensities of the neurons on every image, then results were calculated for the experimental groups. Statistical analysis was performed with SigmaStat software. Conclusion: We detected statistically significant difference (decrease) in PDD samples in both MFG ($p = 0.005$) and midbrain ($p < 0.001$) regions compared to the control group using Mann-Whitney U-test. According to our results the level of LMTK2 is significantly decreased in PDD compared to controls in the brain regions playing a crucial role in cognitive symptoms (MFG) and movement disorders (midbrain). Probably, the protein is implicated in the pathomechanism of PDD providing a potential future therapeutic target in the treatment of the disease.

Keywords: Parkinson's disease – dementia – neurodegeneration – LMTK2

P030

Stimulation of subthalamic nucleus in Parkinson's disease is associated with increased nerve fiber density in the internal globus pallidus

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Introduction: Stimulation of the subthalamic nucleus (S-STN) is an effective treatment for Parkinson's disease (PD). Still, the physiological mechanisms mediating the therapeutic response are poorly understood. Studies in human postmortem tissue are rare. Objectives: Study histological changes associated with S-STN in the internal globus pallidus (GPI), the main target structure of STN efferent fibers, in PD patients. Methods: Postmortem histological samples of GPI from 9 PD patients medically treated (M-PD), 9 PD patients who underwent S-STN and 11 normal controls (CTRL) were analyzed. Using immunohistochemistry, we evaluated fiber density using anti-neurofilament SMI 310 antibody (manual counting) and synaptic terminal density using synaptophysin antibody (semi quantitative assessment). Results: Subjects from all groups had similar age at death ($p = 0.1$) and postmortem delay ($p = 0.3$). Neurofilament immunohistochemistry ($n = 9$ M-PD; $n = 9$ S-STN; $n = 11$ CTRL) showed an increased frequency of axonal endings around GPi neurons' cell body in S-STN subjects (8.54 axonal endings/neuron, SD 2.64) versus M-PD subjects (5.09 axonal endings/neuron, SD 3.14; $p = 0.016$) and CTRL (4.46 axonal endings/neuron, SD 3.76; $p = 0.017$). Preliminary results have shown a tendency of greater synaptophysin expression around cell bodies in S-STN-PD patients ($n = 3$, density value 2.74) compared to M-PD patients ($n = 3$, density value 1.94) and to CTRL ($n = 5$, density value 2.05). A larger cohort is being analyzed. Conclusion: In S-DBS patients, we found increased density of axonal and synaptic terminals surrounding the cell body of GPi neurons. The GPi is the main basal ganglia target of STN glutamatergic axons, suggesting a potential neurotrophic effect of DBS. Further studies to identify the type of neurons involved are underway.

Keywords: Parkinson disease deep brain stimulation

P031

Pathogenic alpha-synuclein is present in the kidneys of Lewy body disease patients

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Background: Parkinson's disease (PD) and dementia with Lewy bodies (DLB) are degenerative disorders of the central nervous system (CNS) with pathological hallmark of phosphorylated alpha-synuclein deposition in Lewy bodies (LBs) and Lewy neurites (LNs). Recently, population-based studies have revealed that patients with kidney diseases, in particular chronic kidney diseases are associated with a higher long-term risk of PD. Objectives: Herein, we sought to determine for the first time whether kidney is another peripheral organ that may serve as an origin of pathologic form of alpha-synuclein to spread to the brain. Methods: Eleven patients with Lewy body disease (LBD), five healthy controls, and seven patients with end stage renal disease (ESRD) were examined for the presence of pathogenic phosphorylated alpha-synuclein at the residue serine 129 (pS129 alpha-synuclein) in kidney and brain. Results: Ten of eleven subjects with PD or DLB had accumulated pS129 alpha-synuclein in their kidneys. Although some of the healthy control kidneys contained detectable pS129 alpha-synuclein, all chronic renal disease patients had prominent alpha-synuclein pathology including LBs and Lewy neurites LNs in the CNS. Conclusion: Our observation has provided the first evidence that the kidney may serve as another peripheral organ of initiation site for pathogenic alpha-synuclein to spread, suggesting compromised renal function might affect the risk of developing LBD.

Keywords: Alpha-synuclein – dementia with Lewy bodies – kidney – Lewy body disease – Parkinson's disease

P032

Disease-linked IFNAR1^{C291*} mutation in familial Parkinsonian disorders leads to mitochondrial dysfunction and proteinopathy

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Introduction: Parkinsonian disorders, including Parkinson's disease (PD), are the second most common class of neurodegenerative disorders. Despite numerous efforts, PD is largely sporadic with unknown aetiology. Nevertheless, studies of rare familial forms have identified genetic mutations– shedding light on the underlying pathophysiology. Conclusion: Here, we report the identification of a novel, nonsense, disease-linked mutation of the interferon a/b receptor 1 (*IFNAR1*) gene, p.(C291*), in a family affected by parkinsonian disorders. Heterozygous expression of the truncated receptor *IFNAR1*^{C291*} in primary neurons and patient-derived fibroblasts had a dominant-negative effect, impairing IFNAR-dependent downstream signalling. Furthermore, IFNAR1^{C291*} expression induced defective neuronal morphology and the pathological hallmarks of PD: accumulation of senesced mitochondria, increased oxidative stress, lower mitochondrial respiration, and intracellular inclusions containing phosphorylated a-synuclein, Tau and ubiquitin. Additionally, neuronal expression of IFNAR1^{C291*} in vivo led to motor and cognitive deficits, dopaminergic neuron loss and the presence of reactive microglia. Our data reveal a new link between innate immunity and Parkinsonian disorders, highlighting new pathogenic mechanisms involved in the etiology of the disease.

Keywords: Neuroinflammation – Parkinson's disease – Type I interferons

P033/SY 7.5

Defining and diagnosing neurodegenerative movement disorders through integrated analysis of genetics and neuropathology (MD-GAP)

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Introduction: Early and accurate diagnosis in Parkinsonian disorders may be difficult in the earliest disease stages. Moreover, disease progression varies from patient to patient. Genetics have provided tremendous insights into neurological diseases, largely based on studies in clinically diagnosed patients. The gold standard for diagnosis is still neuropathology, and this defines the primary protein pathology which is a potential target for future therapy. Purpose: We aim to integrate clinical and genetic data with neuropathology on a large scale to better understand the genetic drivers of the disease process and improve the diagnostic accuracy of movement disorders. We hope to generate a resource to enable secondary analyses which use clinical, pathological and genetic information. Methods: We will use the Illumina GDA with Neurobooster to genotype 2800 neuropathologically confirmed cases with Parkinsonian disorders and 1000 controls in a collaborative project with the UK Brain Bank Network. We will conduct pathologically defined case-control, case-case and progression GWAS, and use the data to develop polygenic risk tools to enhance diagnostic accuracy. In a subset of 750 Lewy body disorder (LBD) cases, we will use digital pathology approaches to quantify the amyloid, tau and alpha-synuclein burden. We will carry out gene and transcript analyses to study the genetic drivers of pathology and integrate this with analyses of clinical heterogeneity to decode the different patterns of neurological disease, which may ultimately respond to different therapies. Conclusion: We believe this study will improve our understanding of the neurobiology of movement disorders and contribute to the development of disease-modifying treatments.

Keywords: Movement disorders – genetics – neuropathology

P034

Regulation of pathogenic gene expression through chromatin alterations in Parkinson's disease

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Introduction: Clinical diagnosis of Parkinson's disease (PD) relies on the presence of motor symptoms, although non-motor symptoms can manifest themselves already up to 20 years earlier. Dissecting molecular changes occurring in the prodromal stages of the disease could facilitate earlier and more accurate diagnosis. Methods: Investigating gene expression signatures and chromatin profiles during disease development of in vivo midbrain sections and isolated nuclei of different cell types from various PD mouse models was established. This process includes staining, nuclei extraction and sorting of different cell populations for ATAC-seq and RNA-seq analysis. Through the integrative analysis, we aim to identify the upstream regulatory events and key transcription factors controlling the pathogenic gene expression changes at the chromatin level. Results: Following this approach, we have observed a robust age- and sex-dependent transcriptomic deregulation in a knock-out mouse model for *Park7* gene, an important PD gene encoding for DJ-1. Some of the affected genes are also misregulated in an overexpression mouse model with human mutated alpha-synuclein transgene, indicating the existence of common changes between the mouse models. Conclusion: Further analysis to validate upstream regulators and pathways predicted by enrichment analysis in *Park7*$^{-/-}$ mice will be performed.

Keywords: Epigenetics – Parkinson's disease – prodromal stages

Animal models-neurodegeneration

P035

Alpha-synuclein-induced pathologies in brain and gut by curli and a fiber-deprived diet in a transgenic mouse model of Parkinson's disease

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Introduction: Parkinson's disease (PD) is a complex, multi-factorial disease for which multiple mechanisms have been postulated to initiate or contribute to its pathology. We hypothesize that PD pathogenesis can be triggered and driven by a combinatorial effect of (1) reduced gut barrier function driven by microbial mucus foraging as a consequence of dietary caused microbiome imbalance, (2) exposure of the enteric nervous system to bacterial amyloidogenic peptides that initiate alpha-synuclein (αSyn) aggregation and prion-like propagation from the PNS to the CNS. Methods: We therefore exposed an overexpressing human wild-type alpha-synuclein transgenic mouse model to a fiber-deprived diet and amyloidogenic protein curli-producing bacteria. Results: Our results suggest that, while behavior is mainly driven by the transgene, the microbiome by the diet and neuropathology by curli, when transgenic animals are exposed to both the fiber-deprived diet and curli producing bacteria, we observe a stronger phenotype. Transgenic animals, which were exposed to both the fiber-deprived diet and the curli-producing bacteria showed decline in movement coordination over time in comparison to all other groups. Neuropathological results show a subtle while conserved pattern of neurodegeneration and aSyn aggregation across different PD relevant areas in transgenic animals exposed to curli-producing bacteria, exacerbated by dietary fiber deprivation. Conclusion: For the next steps, we plan to fully investigate how the enteric nervous system has been altered, further investigate how the microbiome and potential other mechanisms such as the immune system system or metabolites contribute to the observed phenotype.

Keywords: PD – Gut-Brain-Axis – Alpha-Synuclein – Microbiome

P036

Alpha-synuclein and gene expression regulation: insights from in vivo and in vitro models

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Introduction: Alpha-synuclein (α-syn) abnormally aggregates within neurons, nerve fibers or glial cells in the context of neurodegenerative diseases such as Parkinson's Disease (PD), and α-syn gene mutations, duplication and triplication have been associated with familial PD. Understanding the earliest phases of PD is essential toward improved early diagnosis and intervention. Methods: In a previous study, we have found that strongest gene expression changes in ventral midbrain of a genomic α-syn overexpressor preceded axonal degeneration in the striatum by several months. To better understand the relevance of these molecular changes in the development of PD, we are analyzing gene expression changes in vivo, in the ventral midbrain of a variety of α-syn genetic mouse models, and in vitro, in a human neuronal cell line exposed to neurotoxic α-syn moieties. We use RNAseq-based profiling to uncover key molecular events that underlie preclinical PD. To do this, we are focusing our analyses on changes that occur before the onset of overt neurodegeneration. Results: Our in vivo studies demonstrated that gene expression do not seem to correlate directly with expression levels of transgenic α-syn, nor with the promoter driving α-syn overexpression. Furthermore, initial observations indicate that gene expression changes in the midbrain of non-synuclein based PD mouse models will further help understand the earliest phases of PD (see abstract by Helgueta et al., P034). Conclusion: We expect our investigation to make an essential contribution toward the goal of understanding and detection of preclinical PD.

Keywords: Parkinson's disease – alpha-synuclein – mouse models – gene expression

P037/WS 7.3

Unique changes in gene activity in the ventral midbrain precedes dopaminergic degeneration in Parkinson's disease mouse models

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Introduction: Parkinson's disease (PD) often emerges after asymptomatic decades. Up to now, the early molecular events preceding PD phenotype remain to be determined. Methods: To determine if early PD stages are related to a specific molecular signature, we longitudinally analyzed, at 5 months and 15 months of age, i) the motor behavior, ii) the integrity of the nigrostriatal system in association with iii) the transcriptomic profile of midbrain from synuclein-independent LRRK2 $^{R1441C}_{hRC/+}$ and synuclein-based BAC-Tg3($SNCA^{E46K}$), as well as in double mutants models with a deletion of the neuroprotective factor DJ-1. These models display increased number of parameters affected in the catwalk, and subtle decrease in the nigral dopaminergic cell content and in striatal TH/DAT positive terminals. Results: The PDMap Gene Set Enrichment Analysis of the midbrain transcriptome revealed a "dopaminergic transcription pathway" feature to be consistently affected in every model at every time point. This pathway involves the transcription factors FOXA1 and NR4A2 which regulate the expression of the key-dopaminergic metabolism genes *TH, DAT, VMAT2,* and *RET*. Under pathological stress, the expression of these genes showed an age-dependent alternation pattern in several of our PD models. We confirmed our observations in a seeding/spreading alpha-synuclein PD model consisting in an intrastriatal injection of preformed fibrils. Conclusion: In conclusion, our study shows that the dopaminergic transcription pathway is altered in a synuclein-independent manner before neurodegeneration of the nigrostriatal system occurs. Such a finding points to an early and long-lasting fundamental mechanism which may be of high translational relevance to understand the initial steps of PD.

Keywords: Mouse models – Parkinson's disease – neurodegeneration

P038

Assessment of the endotheliocytes to pericytes ratio in the brain capillary wall in rats with experimental dementia

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Background: The question of the vascular damage role in Alzheimer's disease onset and the possibility of vascular regeneration during neurodegeneration continues to be relevant. Purpose: To study the endotheliocytes to pericytes ratio in the brain capillary wall in rats with different models of Alzheimer's type dementia without and after mesenchymal stem cells administration. Methods: The experiment was performed on 74 male WAG rats with 14-day and 28-day nitrite-induced (Nitr-14, Nitr-28) and scopolamine-induced (Scop-14, Scop-28) model of dementia without and after single intravenous mesenchymal stem cells (MSCs) injections (Nitr-14-SC, Nitr-28-SC, Scop-14-SC, Scop-28-SC) (500.000 cells per rat). The control group (gr.C, n = 16) rats received 0.9% sodium chloride solution. The animals were sacrificed on the 14th day after all injections. The brain slices were stained with Congo-red and according to the Einarson's method. Endotheliocytes and pericytes were counted in the brain capillary wall and their ratio (E/P) was calculated. Results: In gr.C the E/P ratio was 1.5. In gr.Nitr-14 and gr.Scop-14 – E/P = 0.7. In gr.Nitr-28 against the background of a reduced number of brain capillaries E/P = 1.26 and in gr.Scop-28 – 0.82. The MSCs administration promoted an increase in the number of endotheliocytes. The E/P ratio in these groups got closer and even exceeded the control values: Nitr-14-SC = 1.8, Nitr-28-SC = 1.3, Scop-14-SC = 2.5, Scop-28-SC = 1.9. Conclusion: In animals with different models of dementia after the brain capillaries damage the wall strengthening mainly due to pericytes was shown. After the MSCs administration, the regeneration of capillaries took place, mainly due to endotheliocytes.

Keywords: Alzheimer's type dementia – scopolamine – nitrite – capillaries – mesenchymal stem cells

P039

MIMO2 (A *Moringa oleifera* leaf derivative): a possible antidote to neurodegeneration

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Introduction: *Moringa oleifera* (MO) leaf has various reports on its medicinal usefulness, including

effect on some neurodegenerative diseases. Vanadium (V), transition metal emitted into the atmosphere during fossil burning and gas flaring, is implicated in various neurodegenerative conditions. Objectives: This study assesses neurotherapeutic properties of a pure compound (MIMO2) isolated from MO leaves against vanadium-induced neurotoxicity. Methods: Cell culture assays (HT22 cell lines) were used to assess the effect of MIMO2 on vanadium neurotoxicity. Two-week old mice were randomly and equally divided into four groups (n = 5), dosed intraperitoneally for 14 days, sacrificed day 15. Groups: Control, vanadium 3 mg/kg (V), MIMO2 10 mg/kg (M10), M10+V. Histology – H&E, Nissl, Black Gold II histochemistry (BGII), immunohistochemistry (microglia, astrocytes, myelin, axons and oligodendrocyte lineage cells (OPCs)) in single and double immunofluorescence. Results: Concurrent administration of MIMO2 and V in HT22 cells resulted in significant reduction of immuno-expression of reactive oxygen species and vanadium-induced DNA damage. In V group, histology showed Purkinje cell degeneration, depletion and focal multiple layering, cerebral gliosis, neuronal clumping and degeneration. Neuropathologies were considerably reduced with M10 administration. Nissl stain showed significant amelioration of vanadium-induced neuronal loss in the CA1 region of M10+V. Cortices showed microglia and astrocytic hyperplasia and hypertrophy in the vanadium group, with significant amelioration in M10+V. BGII showed severe vanadium-induced demyelination, with significant alleviation in M10+V. In brains of vanadium-treated mice only, OPCs with increased NG2 immunolabeling, hypertrophy and bushy, ramified appearance, were observed. Conclusion: MIMO2 displayed good neurotherapeutic activity against vanadium-induced neurotoxicity in vitro and in vivo.

Keywords: *Moringa oleifera* leaves – MIMO2 – vanadium neurotoxicity – neurotherapeutic activity

P040

Replacing deficient microglia in a zebrafish model of a childhood human leukodystrophy as a new therapy

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Introduction: RNaseT2-deficient leukodystrophy is a rare, heritable white matter disorder with infantile onset: Characterized by devastating psychomotor impairments and white matter lesions. Despite its severity, there remain no treatments for this disorder, with only a limited understanding of the mechanisms underpinning pathogenesis. Over the last decade, the zebrafish has emerged as a leading model of this and similar disorders, with their ex-utero development, transparency during embryogenesis and genetic tractability allowing observation of neuropathology in real time. Recent study of the *rnaset2* mutant zebrafish – which recapitulates both the behavioral and white matter abnormalities seen in human patients – has suggested that microglia are the cellular drivers of pathology in this disorder. Methods: In this study, we aimed to establish a protocol for microglial replacement via macrophage transplantation in zebrafish embryos with preclinical therapeutic relevance to RNaseT2-deficient leukodystrophy. We developed a robust macrophage transplantation protocol in zebrafish embryos and assessed transplantation efficacy using confocal microscopy and functional assays. Results: We demonstrate that microglia can be functionally replaced by transplanted macrophages, with transplanted cells expressing microglia-specific markers. Crucially, transplanted microglia are able to ingest apoptotic cells within host brains. Therefore, our work is among the first to develop a robust protocol for immune cell replacement in zebrafish embryos and provides proof-of-principle that transplanted macrophages can differentiate into functional microglia following engraftment in the brain. Conclusion: Our study suggests that immune cell transplantation may be a viable strategy for microglial replacement which can further inform our understanding of the cellular mechanisms of pathology in RNaseT2-deficient leukodystrophy.

Keywords: Leukodystrophy – white matter disorders – macrophage transplantation – microglia

Neuroinflammation

P041

Innate signaling in the CNS prevents demyelination in a focal EAE model

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Introduction: Stimulation of innate receptors has been shown to suppress experimental autoimmune encephalomyelitis (EAE), an MS-like disease in mice. Specifically, targeting Toll-like receptor 9 (TLR9) and NOD-like receptor 2 (NOD2) significantly reduced disease severity. In the present work we have developed a novel focal EAE model to further study the effects of innate signaling on demyelinating pathology. Objective: To investigate the effects of innate signaling on the development of focal brain lesions. Methods: C57BL/6 mice were immunized for EAE and 10 days later they received a stereotactic needle insertion into the corpus callosum (CC) to induce focal lesions. On day 12, AF488-conjugated MIS416, a microparticle compris-

ing ligands for NOD2 and TLR9, or vehicle (PBS) was administered into the cerebrospinal fluid via intrathecal injection to cisterna magna. Mice were sacrificed on day 13 and 16, and brains and draining cervical lymph nodes were collected for histology, flow cytometry and RT-qPCR. Results: Flow cytometry analysis at day 13 showed that MIS416 influenced the composition of CNS infiltrates, increasing myeloid and NK cells and reducing T cells at the lesion site. On day 16 MIS416 had significantly reduced demyelination and infiltration in the CC when compared to the vehicle control. RT-qPCR analysis of microdissected focal lesions showed upregulation of type i and type II interferons, IL-10, Arg-1 and CCL2 in MIS416-treated mice. Conclusion: In the present study, we demonstrate that intrathecal treatment with selected innate ligands alters the composition of cellular infiltrates at the lesion site resulting in amelioration of focal EAE pathology.

Keywords: Focal EAE – demyelination – innate signaling – brain lesions

P042

From a glial tumor to a tumefactive inflammatory demyelinating lesion – presentation during pregnancy

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Introduction: Tumefactive inflammatory demyelinating lesions (TIDL) can mimic brain tumors. The differential diagnosis may be difficult based only on clinical and imaging features. Clinical case: A 31-year-old, 17-weeks pregnant (by ovarian stimulation) woman, with no past medical history, was admitted to the emergency department with focal evolving to bilateral tonic-clonic seizures. She had a two-month history of morning headaches and behavioural changes. Brain-MRI showed two cortico-subcortical large lesions – right anterior parasagittal frontal and left parieto-occipital, suggestive of a glial tumor. She was submitted to a subtotal resection of the frontal lesion. However, neuropathology showed extensive demyelination, with necrotic areas and frequent perivascular inflammatory infiltrates (macrophages and T-lymphocytes) in white matter, as well as cortical astrocytosis and perivascular inflammatory cuffs, with transmural infiltration but without fibrinoid necrosis. CSF, serological and immunological studies (including oligoclonal bands, antineuronal antibodies, anti-AQP4/anti-MOG, ANCA and other systemic autoantibodies), visual evoked potentials, spinal cord MRI and PET were negative/normal. She was treated with steroids and initiated azathioprine post pregnancy. Three anti-seizure drugs managed seizure-control and neuropsychological evaluation revealed multiple domain cognitive dysfunction. With treatment there was involution of the lesions and at 2 years follow-up, she is professionally active, without new symptoms and no new lesions on MRI. Conclusion: Neuropathology excluded the initial diagnosis of a tumor and guided the investigation towards inflammatory demyelinating diseases. However, the etiology remains unknown, like in many TIDL in literature. Hormonal factors can influence the tumefactive presentation of these lesions in pregnancy, a condition that also raises many issues in terms of investigation and treatment.

Keywords: Tumefactive lesion – demyelinating disease – brain tumor – neuropathology – pregnancy

P043

Neuroinflammation, epilepsy and psychological morbidity in patients undergoing surgery for intracranial meningioma

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Purpose: Meningioma is one of the most common types of brain tumor. Most cases are considered benign but despite technically successful operations, we see patients who suffer from severe complications most importantly being epilepsy, depression and fatigue. Neuroinflammation is a probable cause of these complications and can be present because of the tumor and as a side effect after surgery. The current guidelines for treating patients with meningioma does not consider the effect of neuroinflammation nor the complications after surgery. Material: We will include 80 patients undergoing surgery for supra-tentorial meningioma within a period of 3 years. Methods: We measure neuroinflammation with single photon emission computed tomography (SPECT) that detects microglia cells before and after surgery. We study the biology of neuroinflammation in the same patients with analyses from surgical removed tumor- and brain tissue. Our aim is to describe the inflammatory signature with SPECT imaging together with analyses of inflammatory biomarkers in tumor and brain tissue. We then plan to test the hypotheses that neuroinflammation in meningioma patients is statistically associated with epilepsy, depression and fatigue. We test the patients before and after surgery with neuropsychological questionnaires and tests to detect depression and fatigue. Expected results: We recently performed 6 pilot SPECT scan on patients with meningioma. They all showed very high signal of inflammation which strongly support our hypothesis. We expect to show results from our case series of 6 patients and present our method to detect and analyze neuroinflammation.

Keywords: Meningioma neuroinflammation neurosurgery

P044

Exploring the neuroinflammatory response of the motor regions in human motor neurone disease

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Introduction and Purpose: Microglia, the primary immune cells of the CNS are highly involved in the pathogenesis of Motor Neurone Disease (MND). Their activation has been found to correlate with a number of features of disease. However, how microglia contribute to human MND is not currently known. We aim to elucidate the role of immunity in sporadic MND by examining gene expression in post mortem tissue. Methods: Transcriptional analysis was performed using the nanoString Neuroinflammation panel of 770 genes. RNA was extracted from frozen cervical spinal cord anterior horn and motor cortex from MND patients (16 cases per location) and controls (8 cases per location). Findings: In MND cases compared to controls, 128 genes were differentially expressed in spinal cord, with upregulation of genes linked the NF-kB, TREM2, APOE, and phagocytic pathways. Little immune gene expression was observed in the motor cortex. In the spinal cord of MND cases, 46 genes were associated with survival time (similar numbers correlated with longer and shorter survival). In the motor cortex, 47 genes were associated with patient survival, 30 of which were associated with longer survival. The results from frozen spinal cord correlated well with previous results from FFPE tissue and archival RNASeq and microarray data from other laboratories. Conclusion: The spinal cord appears to be a more inflammatory environment compared to the motor cortex. Immunity-associated gene expression pathways differ between control and MND tissues, highlighting the role of immunity MND. Further work will validate these changes using immunohistochemistry allied with tissue microarray technology.

Keywords: Neurodegeneration – neuroinflammation – pathology – transcriptomics

P045

Magnetic resonance imaging correlates of multiple sclerosis immunopathological patterns

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Introduction: Histology reveals that early active multiple sclerosis lesions can be classified into three main inter-individually heterogeneous but intra-individually stable immunopathological patterns of active demyelination (patterns I-III). In pattern I and II, a T-cell- and macrophage-associated demyelination is suggested, with pattern II only showing signs of a humoral immune response. Pattern III is characterized by inflammatory lesions with an oligodendrocyte degeneration. Patterns suggest pathogenic heterogeneity, and we postulated that they have distinct MRI correlates that may serve as biomarkers. Objectives: To analyze the MRI correlates of multiple sclerosis immunopathological patterns. Methods: We evaluated in an international collaborative retrospective cohort study the MRI lesion characteristics of 789 pre-biopsy and follow-up MRIs in relation to their histopathologically classified immunopathological patterns (n = 161 subjects) and lesion edge features (n = 112). Results: A strong association of a ring-like enhancement and a hypointense T2-weighted rim (T2w rim) with pattern I and II, but not pattern III, was observed. Only a fraction of pattern III patients showed a ring-like enhancement, and this was always atypical. Ring-like enhancement and T2w rims colocalized, and ring-like enhancement showed a strong association with macrophage rims as shown by histology. A strong concordance of MRI lesion characteristics, meaning that different lesions showed the same features, was found, indicating lesion homogeneity within individual patients. Conclusion: We provide robust evidence that MRI characteristics reflect specific histological features of MS immune patterns and that ring-like enhancement and T2w hypointense rims might serve as a valuable non-invasive biomarker to differentiate pathological patterns of demyelination.

Keywords: Multiple sclerosis – immunopathological patterns – MRI correlates – ring-enhancing lesions – hypo intense T2-weighted rim

P046

Bornavirus encephalitis in humans differs from what we know in animals

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Introduction: Borna disease virus 1 (BoDV-1) was only recently discovered to cause a fatal encephalitis in humans. The distribution of inflammation in humans seems to differ from the disease in animals. There is, however, a lack of comprehensive quantitative studies to confirm this observation. Objectives: The aim of this study was to provide a topological comparison of BoDV-1 infection in humans and animals to allow conclusions regarding the spreading of the inflammation including the infection route. Methods: Brain autopsy specimens of BoDV-1 infected humans (n = 6) and animals (5 horses, 4 sheep, 4 alpacas) were investigated with 8 up to 27 samples per individual, including hippocampus, thalamus, basal ganglia, cortex, cerebellum, mesencephalon among others. Beside hematoxylin/eosin stain, immunohistochemistry for BoDV-1 (Bo18), CD3 and C20 was used to quantify topography and magnitude of infection between species. For strongly affected regions, immunohistochemistry for CD68, Iba1, GFAP, and RNA-in-situ-hybridization for BoDV-1 was complemented. Results: The limbic system was predominantly affected in all animal brains, while in humans, BoDV-1 mostly involved the basal ganglia and insula cortex. Furthermore, it was notable that for animals, the infection mostly was limited to specific anatomical/functional regions, whereas it was broadly distributed in humans. Conclusion: The different distribution of BoDV-1 lesions in animals compared to humans might reflect different infection routes and provide new information about the chain of events leading to encephalitis.

Keywords: Bornavirus encephalitis comparison human animals

P047

IFNβ, not IFNα, induces a PD-L1hi FoxA1+ T regulatory signature

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Introduction: FoxA1^{+}T$_{reg}$ cells, a distinct population of regulatory T (T$_{reg}$) cells, were identified in multiple sclerosis patients responding to interferon-beta (IFNβ)-treatment. On isolating FoxA1^{+}T$_{regs}$ from inflamed brains during recovery and good IFNβ-responders among multiple sclerosis patients, we have shown that IFNβ-induced FoxA1^{+}T$_{regs}$ suppress brain inflammation via programmed death ligand (PD-L)1. Yet, the molecular regulation of *Pdl1* by IFNβ and FoxA1 in T cells is unknown. Objectives: We aimed to identify the molecular mechanism by which IFNβ and FoxA1 controls PD-L1 expression in T cells. Methods: We used RNA-seq to characterize the global transcriptomic IFNβ-induced cell fate changes in FoxA1^{+}T$_{regs}$. We used molecular and cellular techniques – luciferase reporter assays, electrophoretic mobility shift assays (EMSA), co-IP, FACS – to address the molecular events leading to *Pdl1* gene activation in FoxA1^{+}T$_{regs}$ during cell fate commitment. Results: We report the first transcriptomic profile of human FoxA1^{+}T$_{regs}$ – induced specifically by IFNβ, not IFNα, and defined by robust FOXA1 and PDL1 expression. On identifying the 60-bp minimal *Pdl1* promoter, we determined that FoxA1 binds to a 27-bp segment within it to drive PD-L1 expression synergistically with IFNβ. This activation was potentiated by IFNβ-induced phosphorylation of STAT1/2, allowing them to complex with FoxA1, translocate to the nucleus, and bind *Pdl1*. Conclusion: Our data characterize the key molecular players and essential sequences in the *Pdl1* promoter required for PD-L1 regulation, a central protein for FoxA1^{+}T$_{reg}$ cell fate determination and suppressive function. These hold strong promises as potential therapeutic targets in T cells to achieve anti-inflammatory properties.

Keywords: Regulatory T cell (Treg) – multiple sclerosis (MS) – interferon beta (IFNβ) – PD-L1 – STAT1/2

P048

The effect of siponimod on glial cell functions and viability in vitro

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Introduction: Siponimod is a selective S1P1 and -5 receptor modulator, approved for the treatment of secondary progressive MS, and in a large phase III placebo-controlled, double-blinded, randomized clinical trial, significantly reduced physical and cognitive impairments, as well as brain atrophy. Siponimod has also been suggested to mediate a possible neuroprotective effect. However, the underlying mechanisms are yet to be fully understood. Purpose: Our primary focus is to study the effect of siponimod on glial cell viability, proliferation, morphology, and phagocytosis in primary astrocyte, microglia, and oligodendrocyte precursor cultures. Methods: Mixed primary glial cell cultures are obtained from C57BL/6J mice pups (P1 – P4) and separate glial cell cultures are prepared by the shake-off method. The IncuCyte ZOOM live-cell imaging system and the Fiji plugin TrackMate allowed us to track live cell migration. Proliferation was quantified by measuring DNA synthesis, by incorporating EdU into DNA during active DNA synthesis. Immunocytochemistry allowed us to study the morphology of the cells, and the phagocytic ability was studied by quantification of the fluorescence from internalized

latex beads. Results: Preliminary data showed altered cellular responses following siponimod treatment, indicating that siponimod may be able to dampen the immune response initiated by LPS-stimulation. Siponimod was also found to expand the population of oligodendrocyte precursor cells, which could be involved in the regenerative ability proposed to be mediated by siponimod. These results are valuable in the planning of further in vivo studies.

Keywords: Progressive Multiple Sclerosis Siponimod

P049

Plasticity between FoxP3+Treg and FoxA1+Treg cells

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Introduction: FoxP3$^+$ regulatory T (T_{reg}) cells and FoxA1$^+T_{regs}$ are key in suppressing autoimmunity. However, the function of FoxP3$^+T_{regs}$ is compromised in multiple sclerosis (MS), but proposed to be restored by IFNβ. On the other hand, MS patients' positive response to IFNβ therapy is associated with generation of anti-inflammatory FoxA1$^+T_{regs}$. We hypothesized that IFNβ induces plasticity in FoxP3$^+T_{regs}$ by FoxA1$^+T_{reg}$ cell programming. Objectives: We aimed to investigate if FoxP3$^+T_{regs}$ and FoxA1$^+T_{regs}$ belong to distinct T_{reg} lineages, and if IFNβ influences plasticity of FoxP3$^+T_{regs}$ by resuming the genetic signature and features of FoxA1$^+T_{regs}$. Methods: We used a high-throughput approach, combining RNA-seq, ChIP-seq, and mass-spectrometry with FACS analysis of human FoxP3$^+T_{reg}$ subsets to identify the global changes that are induced by IFNβ. Results: FoxP3$^+T_{regs}$ and FoxA1$^+T_{regs}$ constitute distinct lineages of T regulatory cells. All of the various human FoxP3$^+T_{reg}$ subsets showed plasticity and acquired the FoxA1$^+T_{reg}$ transcriptomic, genetic, and phenotypic signatures on treatment with IFNβ, including PD-L1 expression. IFNβ induced ISG15 and the ISGylation of FoxA1, the lineage-specifying transcription factor of FoxA1$^+T_{regs}$. FoxA1 upregulated *FOXA1*, *PDL1*, *TRAIL*, *STAT2*, *CD69*, *CD2*, *CD48,* and *ISG15* – the FoxA1$^+T_{reg}$ signature genes – in FoxP3$^+T_{regs}$. Finally, FoxA1 bound to enhancer regions of *FOXP3*, and to *TGFB1/3,* and *TGFBRI,* effecting the repression of TGFβ signaling in FoxP3$^+T_{regs}$. Conclusion: FoxP3$^+T_{regs}$ and FoxA1$^+T_{regs}$ constitute distinct T_{reg} cell lineages. FoxP3$^+T_{regs}$ acquire a FoxA1$^+T_{reg}$ transcriptomic, genetic, and phenotypic signature by IFNβ signaling. Our study is the broadest analysis of the human T_{reg} profile to date and serves as an excellent resource for understanding T_{reg} biology.

Keywords: Regulatory T cells (Treg) – Multiple sclerosis (MS) – interferon beta (IFNβ) – T cell plasticity – Treg signature

P050

Histopathological analysis of T-cell infiltration and chemokine at the human brain infarcts

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Introduction: Immune response of T cells in ischemic brain infarcts has been focused on, and new insights such as the behavior of regulatory T cells (Treg) and its chemotaxis are gained by rodent models. However, less has been described about this behavior in humans. Objectives: This study aims to clarify the chemotactic behavior of T cells including Treg in human brain infarcts. Methods: Using immunohistochemistry and immunofluorescence, we analyzed the distribution of T cells (CD3$^+$) including Treg (FoxP3$^+$) and expression pattern of T cell-chemokines and its receptors in human 14 autopsied samples and 18 surgical samples of variating ischemic changes. Results: In the coagulation necrosis, mild infiltration of CD3$^+$-CCR7$^+$ cells and expression of CCL19 in astrocytes were noted in a peri-infarct area with few FoxP3$^+$ cells. Infiltration of CD3$^+$-CCR7$^+$ cells was evident in the liquefactive necrosis, and peri-infarct area of liquefactive necrosis and cystic lesions. FoxP3$^+$-CCR6$^+$ cells were focally noted near the boundary of necrosis. CCL19- and CCL20-expression was observed in the reactive astrocytes of peri-infarct regions, whereas CCL19-dominant expression in foamy macrophages of infarcted areas. In addition, the accumulation of CCL20 at the rim of vacuoles near the border of necrosis was noted, showing co-localization with several astrocytic markers. Conclusion: The chemotaxis behavior of T cells could vary between times and regions of human brain infarct, by altering expression of chemokines in reactive astrocytes, microglia and foamy macrophages. CCL20 from reactive astrocytes could accumulate near the border of necrosis and contribute to the distribution of Treg in infarcts.

Keywords: Brain infarct – T cell – chemotaxis – astrocytes

P051

Extracellular vesicles from plasma of hyperammonemic rats induce neuroinflammation and motor incoordination in control rats

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Background: The onset of minimal hepatic encephalopathy (MHE) is associated with changes in the peripheral immune system which are transmitted to the brain, leading to neuroinflammation, which leads to cognitive and motor impairment. The mechanisms by which changes in the immune system induce cerebral alterations remain unclear. Extracellular vesicles (EVs) seem to play a role in this process in certain pathologies. Objectives: This work aimed to assess whether EVs play a role in the induction by chronic hyperammonemia of neuroinflammation in cerebellum and motor incoordination. Methods: We isolated EVs from plasma of control or hyperammonemic rats and characterized the differences in protein cargo by proteomics and Western blot. We assessed whether intravenous injection of EVs to control rats induces neuroinflammation in cerebellum and motor incoordination as found in hyperammonemic rats. We analyzed, by immunohistochemistry and Western blot, the effects on glial activation, neuroinflammation, TNFα, TNFR1, NF-κB in microglia, glutaminase, GAT3 in cerebellum and motor coordination. Results: Hyperammonemia increases EVs amount and alters their protein cargo. Differentially expressed proteins are mainly associated with immune system processes. TNFα and its receptor TNFR1 are increased in EVs in hyperammonemia. Injected EVs enter Purkinje neurons and microglia. Injection of EVs from hyperammonemic, but not from control rats, induces motor incoordination, mediated by neuroinflammation, microglia and astrocytes activation and increased IL-1β, TNFα, TNFR1, NF-κB in microglia, glutaminase and GAT3 in cerebellum. Conclusion: Plasma EVs from hyperammonemic rats carry molecules necessary and sufficient to trigger neuroinflammation in cerebellum and the mechanisms leading to motor incoordination.

Keywords: TNFα – TNFα receptor TNFR1 – glial activation – hepatic encephalopathy

P052

SIGLEC1 (CD169): a marker of active neuroinflammation in the brain but not in the blood of MS patients

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Background: SIGLEC1 expression has been used as a surrogate marker for type I interferon activity in autoimmune diseases as well as interferonopathies and viral infections. Objective: We aimed to evaluate SIGLEC1 (CD169) as a biomarker in Multiple Sclerosis and Neuromyelitis optica spectrum disorder (NMOSD) and to evaluate the specificity of SIGLEC1$^+$ myeloid cells for demyelinating diseases. Methods: We used flow cytometry to measure expression of SIGLEC1 on monocytes in 86 Multiple Sclerosis patients, 41 NMOSD patients and 31 healthy controls. Additionally, we histologically evaluated the presence of SIGLEC1+ myeloid cells in acute and chronic Multiple Sclerosis brain lesions as well as other inflammatory and noninflammatory CNS pathologies. Results: We found elevated SIGLEC1 expression in 16/86 (18.6%) Multiple Sclerosis patients and 4/41 (9.8%) NMOSD patients. Patinets with high levels of SIGLEC1 received nearly all exogenous interferon beta as an immunomodulatory treatment and only a small fraction of patients without interferon treatment had increased SIGLEC1 expression. In the CNS SIGLEC1+ myeloid cells were numerous in active Multiple Sclerosis lesions as well as in a range of other acute CNS pathologies of the central nervous system, but not chronic Multiple Sclerosis lesions. Conclusion: In our cohort, SIGLEC1 expression on monocytes was – apart from those patients receiving interferon treatment – not significantly increased in patients with Multiple Sclerosis and NMOSD, nor were levels associated with more severe disease. The presence of SIGLEC1+ myeloid cells in brain lesions could be used to investigate the activity in an inflammatory CNS lesion.

Keywords: Multiple Sclerosis SIGLEC1 neuroinflammation autoimmune

P053

Temporal and spatial patterns of inflammation and oxidative injury in traumatic human spinal cord injury (SCI)

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Background: Post-traumatic inflammatory reaction plays a central role in the expansion of tissue damage in SCI. Limiting bystander damage has been proven beneficial in preclinical studies however, translation into clinical application has failed so far. Objective: To characterize the inflammatory response and extent of oxidative injury in traumatic SCI. Methods: We investigated 22 SCI patients and 5 controls. Samples were collected at different time points post injury. We performed immunohistochemistry on FFPE tissue to characterize the origin and functional states of microglia/macrophages, presence of different lymphocyte subpopulations and extent of oxidative injury. Results: In the SCI lesion-center, microglia was diminished and lost the homeostatic phenotype. During lesion maturation, most myeloid cells in the lesion-center were derived from blood-borne macrophages. They expressed a pro-inflammatory phenotype and a fraction reached an intermediate (pro/anti-inflammatory) activation status. Oxidized phospholipids were initially present in the SCI lesion-center and declined over time. In contrast in the lesion rim, a substantial proportion of myeloid cells was derived from microglia. They showed a strong proinflammatory polarization, although in a short interval a minority of myeloid cells expressed an intermediate activation status. Oxidized phospholipids increased over time and stayed significantly elevated up to months post injury. Overall, lymphocyte count was low and mainly consisted of $CD8^+$ T cells. In single cases plasma cells were observed as well. Conclusion: Our study provides the first systematic description of temporal and spatial patterns of inflammation and oxidative injury in human SCI. These valuable insights will help to adjust future therapy designs.

Keywords: Oxidative injury – adaptive immunity – microglia – blood-derived macrophages – spinal cord injury

P054

Neuropathology and magnetic resonance imaging characteristics in autoimmune glial fibrillary acidic protein meningo-encephalomyelitis

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Background: Glial fibrillary acidic protein (GFAP) meningo-encephalomyelitis is an autoimmune disease of the CNS, associated with antibodies against GFAPα. Clinical manifestations include encephalopathy, abnormal movements, seizures and autonomic dysfunction. Brain MRI may show perivascular radial gadolinium enhancement in the white matter. Since GFAP is a cytosolic protein, pathogenicity of circulating anti-GFAP autoantibodies has not been clarified yet. Objectives: To determine type and extent of inflammatory nervous tissue injury, which may shed light on the pathogenesis of the disease. Methods: First report of an anti-GFAP meningo-encephalomyelitis case with in-vivo and post-mortem-MRI as well as (immuno-) histochemical staining on routine and double-hemispheric-FFPE brain sections. Results: A 75-year-old woman presented with hallucinations, disorientation, rigor of the upper extremities and tetra-ataxia. In-vivo MRI showed bilateral periventricular and hypothalamic hyperintensities and linear gadolinium enhancement in the basal ganglia. Neuroimmunological work-up revealed anti-GFAPα antibodies in the CSF in tissue and cell-based assays. Despite steroid treatment, the patient died from cardio-respiratory failure. Post-mortem 7 Tesla-MRI of FFPE brain tissue showed prominent perivascular hyperintensities in the white matter that neuropathologically corresponded to dilated Virchow-Robin-spaces with abundant $CD20^+$, $CD79a^+$, and $CD4^+$ lymphocytic infiltrates. $CD8^+$T cells were sparse. GFAP immunohistochemistry revealed an extensive subpial band-like gliosis and reactive astrocytes in the deep cortical sulci in topographical association with meningeal inflammation. In addition, moderate Alzheimer-associated neurodegenerative changes were observed. Conclusion: Neuropathology suggests a crucial role of B and $CD4^+$ T cells in the pathogenesis of anti-GFAP meningo-encephalomyelitis. Perivenous inflammation and dilated Virchow-Robin spaces are plausible pathological correlates of linear gadolinium enhancement in-vivo.

Keywords: GFAP – autoantibodies – meningo-encephalomyelits – MRI – neuroinflammation

P055

Biopsy proven cerebral sparganosis: a case report

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Introduction: Cerebral sparganosis is a rare parasite central nervous system infection. The clinical manifestation is heterogeneous and difficult to diagnose. Sometimes neuroimaging could mimic brain tumors. Although discovery of Spirometra mansoni during operation is standard

for diagnosis, it is not so easy to seen. Objectives: To discuss the neuroimaging and histology characteristics of cerebral sparganosis for diagnosis. Methods: We reported a case of cerebral sparganosis with a series of neuroimaging and pathology after operation. Histology included H&E and immunohistochemical stains. Results: A 28-year old female patient presented with weakness and numbness of right hand for 1.5 months. After admission, brain MRI revealed an occupying lesion at left frontal and temporal lobe, with severe surrounding edema and multiple ring-like enhancements. Brain tumor or inflammatory granuloma was suspected. Serum and CSF examination revealed no definite evidence. Exploratory craniotomy was done and lesion resection was performed. By microscope, necrosis and a wide spread of inflammatory cells were seen. There were granulomas formation and eosinophils around. No pathogen was found. The histology indicated parasite infection. Recurrent serum examination showed positive antibody of Spirometra mansoni. After several courses of praziquantel treatment, the patient recovered well. Conclusion: Diagnosis of rare parasite infection is difficult. Necrosis and granuloma with eosinophils in histology suggested parasite infection, but it's difficult to find pathogens.

Keywords: Cerebral sparganosis – eosinophils – granuloma

P056

Rifaximin prevents motor incoordination in rats with mild liver damage by preventing immune cell infiltration and neuroinflammation in the cerebellum

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Introduction: Patients with liver cirrhosis may show minimal hepatic encephalopathy (MHE), with mild cognitive impairment, psychomotor slowing and motor incoordination, which reduce life quality and span. MHE onset is associated with a shift in peripheral inflammation that would promote infiltration of lymphocytes into the brain. Cerebellum of patients with steatohepatitis show T-lymphocytes infiltration and neuroinflammation suggesting that the changes triggering MHE may already occur at early stages of the liver disease. Rifaximin is a non-absorbable antibiotic administered to patients with MHE, which improves neurological function. Objectives: The aims of this work were to assess if rats with mild liver damage show motor incoordination and if it is associated with changes in cerebellar neurotransmission, neuroinflammation and the immune system; and to assess if rifaximin prevents the changes in the immune system and normalizes neuroinflammation and neurotransmission and improves motor coordination. Methods: Mild liver damage was induced by CCl_4 injection during 4 weeks. Immune cells infiltration and neuroinflammation in the cerebellum were assessed by immunohistochemistry. The content of extracellular GABA and glutamate and of its transporters was analyzed. Motor coordination was assessed in the Rotarod. Results: TNFα, CCL20, CCL2, and CX3CL1 increased in cerebellum promoting T-lymphocytes and macrophages infiltration and neuroinflammation. Mild liver damage altered neurotransmission in cerebellum and induced motor incoordination. Rifaximin prevents immune cells infiltration, neuroinflammation, alterations in neurotransmission and motor incoordination. Conclusion: These data show that mild liver damage induces immune cell infiltration in cerebellum and motor incoordination. This report provides new clues on the mechanisms of the beneficial effects of early treatment with rifaximin.

Keywords: Minimal hepatic encephalopathy – cerebellum – neuroinflammation

P057

The serine protease HTRA1 increases in the CSF in Multiple Sclerosis and correlates with disease progression and disability

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Background: *High Temperature Requirement Serine Protease A1 (HTRA1)* is an enzyme involved in ECM degradation, proliferation, migration and phagocytosis. HTRA1 is present in the CNS, though not much is known about its function. HTRA1 has been suggested to support neural outgrowth and astrocyte development while modulating immune responses. HTRA1 is implicated in CNS diseases, such as CARASIL and Alzheimer's disease, but has not previously been linked to Multiple Sclerosis (MS). Methods: We investigated the levels of HTRA1 in the CSF of patients with clinical isolated syndrome (CIS) (n = 24), as well as relapsing-remitting (RRMS) (n = 23) and secondary progressive MS (SPMS) (n = 26) using ELISA before and after treatment with disease modifying therapies (DMTs). Cellular distribution in human brain tissue was studied using immunochemistry and the oligointernode database. Results: HTRA1 was significantly elevated in RRMS and SPMS patients

compared to healthy controls (HCs) ($p < 0.0001$) and DMT treatment significantly decreased HTRA1 levels in both types of MS ($p < 0.0001$ and $p = 0.0044$). EDSS at baseline and after treatment correlated with CSF levels of HTRA1 ($p = 0.0003$ and $p = 0.0004$). HTRA1 cut-offs were able to discriminate HCs from RRMS patients with 100% specificity and 82.6% sensitivity. In the brain, HTRA1 was expressed in different types of glia and in neurons and the cellular distribution was increased in the progressive MS brain. Conclusion: HTRA1 is a promising CSF biomarker for MS correlating with disease- and disability progression. Most cell species of the normal and diseased CNS express HTRA1 and the expression pattern could reflect pathological processes involved in MS pathogenesis.

Keywords: HTRA1 – biomarker – multiple sclerosis – cerebrospinal fluid – neurodegeneration

P058

Autoimmune global hippocampal amnesia as a manifestation of AMPAR encephalitis and associated neuropathological findings

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Background: Anti-AMPA receptor (AMPAR) encephalitis is an antibody-mediated autoimmune encephalitis which usually manifests with limbic encephalitis, psychosis, and epileptic seizures. Objectives: To report a novel clinical phenotype of AMPAR encephalitis and provide neuropathological description of one autopsy case. Methods: Retrospective review of patients with AMPAR antibodies, comprehensive cognitive and neuropsychological assessment, antibody testing by in-house tissue-based assay as well as in-house cell-based assay, and neuropathological analysis of brain autopsy tissue including histology and immunohistochemistry. Results: We identified three patients with acute to subacute onset of global amnesia. Cognitive performance, attention, concentration and verbal function was not affected and patient symptoms were mimicking transient global amnesia in onset albeit with longer duration. Patients showed no behavioral changes or psychiatric symptoms, and no epileptic seizures or overt inflammatory CSF or MRI changes. Complete remission was seen in two patients after immunotherapy. One patient only partially responded to immunotherapy and died 3.5 months after disease onset due to tumor progression of an adenocarcinoma of the lung. Neuropathological analysis of the brain revealed hippocampal sclerosis (ILAE type 1) with microglial activation, astrogliosis, and mild to moderate meningeal, parenchymal and perivascular inflammatory infiltrates. Parenchymal infiltrates were predominantly composed of CD3/$CD8^+$ T-cells. B-cells and plasma cells were mainly restricted to the meninges. AMPAR-immunoreactivity was bilaterally decreased in the patient's hippocampal formation. Conclusion: AMPAR-antibodies usually associate with limbic encephalitis, but may present with immune responsive, subacute, selective hippocampal dysfunction. Neuropathological investigation showed hippocampal sclerosis with T-cell dominated inflammatory involvement of the CA1/CA4 region and decreased expression of AMPA receptors.

Keywords: Autoimmune disease – AMPAR encephalitis – anti-AMPAR antibodies – gobal amnesia – hippocampal sclerosis

P059

Paraneoplastic cerebellar degeneration with anti-P/Q-type VGCC and anti-Yo autoantibodies: a comparative study

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Introduction: Paraneoplastic cerebellar degeneration (PCD) is characterized by a widespread loss of Purkinje cells (PC) and can be associated with autoantibodies against intracellular antigens such as Yo or cell surface neuronal antigens such as the P/Q-type voltage-gated calcium-channel (VGCC). While the intracellular location of the target antigen in anti-Yo-PCD supports a T

cell mediated pathology, the immune mechanisms in anti-VGCC-PCD remain unclear. Aim: To compare neuropathological characteristics of PCD with anti-VGCC and anti-Yo autoantibodies. Methods: We performed neuropathology and immunohistochemistry on FFPE brain tissue of one anti-VGCC and two anti-Yo-PCD autopsy cases. Results: Anti-Yo-PCD revealed a diffuse and widespread loss of PCs together with microglial nodules, CD8+ T cell infiltrates and upregulation of MHC class I molecules in remaining neurons. In contrast, PC loss in anti-VGCC-PCD predominantly affected the upper vermis, while caudal regions and lateral hemispheres were spared. Inflammation was characterized by only scattered CD8+ T cells and single perivascular B cells, mainly within the cerebellar white matter. No complement deposition or MHC class 1 upregulation on PCs was detected. In both PCD types, calbindin expression was reduced or lost in remaining PCs compared to controls. Conclusion: Anti-Yo-PCD showed characteristic features of a T cell mediated pathology, while this was not observed in anti-VGCC-PCD. Our findings support a pathogenic role of VGCC antibodies in causing neuronal damage, probably due to altered synaptic transmission resulting in calcium dysregulation and cell death. Since disease progression may lead to irreversible PC loss, anti-VGCC-PCD patients could benefit from early oncologic and immunologic therapies.

Keywords: Neuroimmunology – autoantibodies – Yo antigen – P/Q-type VGCC – paraneoplastic cerebellar degeneration

P060

Diagnostic conundrum in a rare case of isolated CNS lymphomatoid granulomatosis

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Introduction: Lymphomatoid granulomatosis (LG) is a rare, Epstein-Barr virus (EBV) associated lymphoproliferative disease, most frequently affecting lungs. The central nervous system (CNS) is affected infrequently. Isolated or primary CNS LG is very rare. A recent systematic review identified less than 50 cases reported in the literature. Case report: A 60 year-old female with history of myasthenia gravis and previous thymectomy for thymoma, in an immunocompromised state, presented with CNS symptoms, with multiple cerebral lesions. She had three brain biopsies before a definitive diagnosis was established. Histopathology: All biopsies showed similar features with necrotising, T-lymphocyte rich, destructive chronic inflammation, without granuloma formation or evidence of active demyelination. Primary CNS neoplasm and lymphoma was excluded by multiple expert review and extensive immunohistochemistry work up. A demyelinating and infectious process was also excluded. The final biopsy revealed a proportion of atypical B-lymphocytes and EBV expression. These features combined with predominance of T-cells, angiocentric pattern of infiltrates and necrotising process prompted a diagnosis of LG. Discussion: The clinicopathological features of isolated CNS LG remains to be fully elucidated. The diagnosis relies mainly upon biopsy. The common mimic is a lymphoma, albeit LG is a risk factor for EBV-driven diffuse large B-cell lymphoma. The prognosis is variable and treatment includes surgery, steroids, chemotherapy and radiotherapy. Conclusion: We suggest considering LG in inflammatory brain biopsies, even in unsuspected cases and to have a low threshold for haematopathology review.

Keywords: Lymphomatoid granulomatosis inflammatory brain biopsy

P061

Neuronal diversity in the multiple sclerosis brain by single-nuclear RNA sequencing

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Background and Objectives: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system. Besides demyelination, MS inflammation damages neurons that results in permanent clinical disability. Currently, this neurodegenerative process cannot be prevented by modern disease modifying therapies. Morphometric and transcriptional studies in MS brains found neuronal loss in superficial cortical layers, however, a substantial portion of neurons seems to be able to survive in the hostile milieu. Here, we performed a high-depth transcriptional analysis of single neurons from variably lesioned gray matter in MS by full-length single-nuclear RNA sequencing (snRNA-seq), in order to identify and study neuronal subtypes and their differences in vulnerability. Methods: We collected and morphologically characterized lesioned

postmortem cortical samples from MS patients and age, sex and brain area matched controls that were not affected by neurological diseases. We sorted NeuN-positive neuronal nuclei into 96-well plates from each sample and performed snRNA-seq based on an optimized Smart-seq2 protocol. Single nuclei were multiplexed and sequenced at an average depth of one million reads per nuclei. Results and conclusion: Unbiased hierarchical integrative clustering identified 11 excitatory and 5 inhibitory, neuronal clusters which could be assigned to well-described subtypes across all cortical layers. Subtype-specific numerical loss of neurons was not observed in our investigated cohort. Yet, we noted profound changes in the expression of inflammatory, apoptotic and synaptic signatures at selected neuronal clusters that may indicate their differential vulnerability. Untangling the molecular underpinnings of such differences in MS could help to identify new neuroprotective targets.

Keywords: Multiple sclerosis – neurodegeneration – snRNA-seq

P062

The extent and distribution of fibrinogen deposition in progressive MS correlate with meningeal and choroid plexus inflammation and lesion activity

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Introduction: Numerous evidence suggest key role of fibrinogen in glia alterations and neurodegeneration in progressive multiple sclerosis (MS). Objectives: We aimed to better understand expression/localization of fibrinogen in post-mortem MS brains with particular attention to subarachnoid/subpial and subependymal/periventricular locations. Methods: Using immunohistochemistry/immunofluorescence the extent/distribution of fibrinogen was examined in grey- (GML) and white-matter lesions (WML) and normal-appearing areas from post-mortem MS cases (30), healthy controls (5), and other neurological conditions (5). In addition, specific correlations with inflammatory correlates were examined in meningeal, choroid plexus, perivenular infiltrates and cerebrospinal-fluid (CSF). Results: We found "pial-in" and "ependymal-in" decreasing gradient of fibrinogen deposition in MS brains, in particular in the thalamus and hippocampus, associated with high expression of fibrinogen in meninges and in choroid plexuses, as well as in active lesions. Fibrinogen deposition was found: On MAP2+neurons and GFAP+astrocytes in GML; on astrocytes and MHC-class II+ microglia/macrophages in WML. Significant higher levels (fold change = 3, $p = 0.01$) of fibrinogen were detected in CSF of MS patients compared to controls. CSF levels of fibrinogen were found significantly associated ($r = 0.76$, $p < 0.05$) with increased frequency of early and late active MS lesions and with both elevated density of CD163+ microglia/macrophages ($r = 0.73$, $p = 0.004$) infiltrating meninges and sub-ependymal vessels and with CSF levels of sCD163 ($r = 0.72$, $p = 0.012$). Conclusion: Increased CSF levels and extensive brain deposition of fibrinogen are associated with meningeal and choroid plexus inflammation and substantial activity in adjacent subpial and subependymal lesions. These findings support a key role of fibrinogen in surface-in MS-brain pathology, possibly by inducing and/or exacerbating microglia activation.

Keywords: Multiple sclerosis – fibrinogen – meninges – choroid plexus – inflammation

P063

Substantial "ependymal-in" gradient of thalamic damage in progressive multiple sclerosis

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Introduction: Leptomeningeal inflammation was demonstrated associated with subpial 'surface-in' gradient of damage in multiple sclerosis cortical grey matter. Aim: We asked whether a similar gradient of damage occurs in deep grey matter thalamic nuclei as an effect of intrathecal/subependymal inflammation. Methods: We examined dorsomedial thalamic nuclei tissues and paired CSF samples from 41 post-mortem secondary progressive MS (SPMS) cases, counting MHC-classII+ microglia/macrophages and NeuN+ neurons in 10 SPMS cases with meningeal tertiary-lymphoid-like structures(TLS+), 10 SPMS cases without meningeal TLS(TLS-) and 5 controls. Results: Thalamic demyelination was observed in 90% of cases, and active lesions in 40%. MS cases exhibited significant reduction in NeuN+ neuronal density compared to controls, and this damage exhibited a CSF-oriented gradient, greatest (26%) in subventricular locations with respect to 12% at 10 mm from the ependyma/CSF boundary. The gradient was reversed for MHC-II+ microglia density, which was increased by over 50% at 2 mm from the ventricular surface vs 15% at 10 mm. Most of the periventricular MHC-II+ cells (70%) expressed the resident-homeostatic marker TMEM119. These gra-

dients were greatest in TLS+ cases and were associated with: Early age at onset and at death; increased CSF levels of Nf-L, chitinase-3-L1, sTNFR1, TNF, CCL19, CCL21, CCL22, CXCL10, CXCL13, IFNγ, sCD163, fibrinogen, IL2, and IL10; perivascular sub-ependymal infiltrates containing abundant CD20+B cells, CD3+T cells, plasma cells, scattered CD35+follicular-dendritic cells and CXCL13. Conclusion: Increased thalamic pathology is associated with more severe progressive MS and occurs according to ependymal-in gradient accompanied by presence of elevated inflammation, compartmentalized in sub-ependymal lymphoid-like perivascular infiltrates and in CSF.

Keywords: Multiple sclerosis – thalamus – gradient of damage – inflammation – cerebrospinal fluid

P064

NRF2, a promising target to reduce microglial reactivity in PD

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Introduction: Microglia constituting a complex population of brain immune cells act as the first line of defense to all types of CNS disorders. A microglial activation can however further increase tissue damage and negatively impact disease outcome. The age-related motor disorder, Parkinson's disease (PD), is characterized by a progressive loss of dopaminergic neurons in the pars compacta of the Substantia Nigra and the presence of proteinaceous insoluble inclusions called Lewy bodies containing high concentration of α-synuclein (α-syn). Thus, microglial reactivity, induced by extracellular α-syn, might contribute to this dopaminergic neuron depletion. Methods: We previously described that the A53T mutant α-synuclein* promotes a pronounced pro-inflammatory phenotype on primary microglia. Here we focused our attention on the effects of a pharmacological activation of the NRF2 signaling pathway by treating our mouse primary microglial cultures with apomorphine, a chiral molecule currently used for PD treatment. Results: The obtained results provided evidence that, through the activation of the NRF2 signalling pathway, apomorphine enantiomers decrease A53T-induced microgliosis, leading to a lower pro-inflammatory state and restoring the phagocytic activity. Suppressing NRF2 recruitment abolished the anti-inflammatory activity of apomorphine. Conclusion: In conclusion, the recruitment of the NRF2 transcription factor may be suitable to decrease α-synuclein-induced microglial reactivity.

*The substitution of alanine (A) to threonine (T) at position 53 of the α-syn protein (A53T) was identified as the cause of a severe autosomal dominant trait of Parkinsonism, characterized by an early onset with a short disease duration to death (less than 10 years).

Keywords: Mouse – microgliosis – alpha-synuclein – NRF2 transcription factor

P065

Neuronal activation and modulation of necroptosis signaling in progressive multiple sclerosis

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Background: Transcriptomics and protein analysis of cortical GM in progressive MS suggests a pivotal role for TNF/TNFR1 activation of necroptosis, involving the phosphorylation of MLKL. The ESCRTIII machinery antagonises necroptosis by generating bubbles that shed MLKL. Objectives: To explore the role of sustained production of cytokines in the meninges as a trigger for the activation of TNFR1-dependent necroptosis and downregulation of the ESCRT-III pathway, leading to neurodegeneration in progressive MS. Material and methods: Quantification of protein and mRNA levels was carried out on isolated cortical GM from post-mortem MS brains and controls by western blotting, immunohistochemistry and QRT-PCR. A rat model of meningeal inflammation was used to determine the consequences of elevated CSF cytokines on cortical neurons. Results: A significant increase in protein levels for TNFR1 and the subsequent key steps of necroptotic signaling, namely the RIPK1, pRIPK3 and pMLKL cascade, was present in neurons in the MS GM, predominantly in layers II-III and V-VI. pMLKL levels were higher in MS cases with more abundant meningeal inflammation. Expression of components of the ESCRT-III machinery were downregulated in the MS GM and correlated inversely with TNF and MLKL levels. These changes were reproduced in the rat cortex by chronic overexpression of TNF and IFNg in the meninges, which gave rise to significant neuronal loss in cortical layers II-III due to necroptosis. Conclusion: Taken together, our data suggest that neurons in the MS cortex are dying via TNF/TNFR1 stimulated necroptosis, possibly initiated by chronic meningeal inflammation and downregulation of the ESCRT-III pathway.

Keywords: Multiple Sclerosis – neurodegeneration – necroptosis

P066

Immunotherapy induced hypophysitis: a case report

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Background: A 30-year-old lady with BRAF V600E-mutant melanoma, receiving her third cycle of Ipilimumab and Nivolumab immunotherapy, presented with headaches and nausea. Objectives: Magnetic resonance imaging of the pituitary region showed a large contrast-enhancing pituitary mass with suprasellar extension, and small enhancing lesions in the left frontal and parietal lobes. The radiological impression was that of a metastasis. The patient underwent resection of the pituitary lesion. Methods: Macroscopically, the specimen comprised of nodular cream and haemorrhagic tissue fragments, together 11×8×5 mm. The sample was embedded in its entirety and routine Haematoxylin & Eosin staining as well as special stains and immunohistochemistry (IHC) was performed. Results: Histology showed non-neoplastic anterior pituitary glandular tissue with preserved architecture and a heterogeneous population of neuroendocrine cells. There was subtle focal lymphocytic inflammation confirmed on CD45 IHC. There was no melanoma metastasis based on morphology and a panel of melanoma markers. Conclusion: This pituitary resection specimen showed no evidence of a melanoma metastasis based on the material examined. There was focal lymphocytic inflammation consistent with a lymphocytic hypophysitis. This case represents an example of lymphocytic hypophysitis arising as a complication of treatment with immunotherapy. Immunotherapy-induced hypophysitis is reported to occur in 0.5 – 22% of patients and is an important consideration when examining presumed pituitary metastases from patients on immunotherapy. As in this case, the inflammatory component may be subtle.

Keywords: Pituitary – hypophysitis – immunotherapy

P067

Antibodies to MOG in CSF only: pathological findings support the diagnostic value

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Introduction: Serum myelin oligodendrocyte glycoprotein antibodies (MOG-Abs) define a distinct entity of inflammatory CNS diseases, presenting with optic neuritis and/or myelitis in adults and encephalomyelitis in children. Atypical fulminant cases have been described. Recent data support the diagnostic value of testing CSF in suggestive seronegative cases. Scarce pathological evaluations confirmed the distinct entity of MOG-Abs associated disorders. Objective: We report the first brain and spinal cord autoptic findings of a patient with MOG-Abs in CSF only. Background: A 81-year-old man presented with urinary retention followed by paraplegia and L1 sensory level loss. Spinal cord MRI showed multiple T2 hyperintense lesions in brainstem and spinal cord, a tumefactive central lesion extending from T9 to the conus, meningeal enhancement along the surface of brainstem, spinal cord and nerve roots. CSF analysis revealed increased protein content and mild lymphocytic pleocytosis. Investigations for infectious and hematological conditions yielded negative results. CSF only MOG-Abs were detected. Despite treatment with high-dose intravenous steroids, the clinical picture rapidly deteriorated. The patient died 15 days after symptoms onset. Results: Pathological examination showed extensive demyelination in pons and cervical spinal cord with mononuclear cells infiltration in the parenchyma and leptomeninges. Plaques were characterized by confluent demyelination with massive accentuation in perivascular spaces and along the circumference of spinal cord. We observed relative preservation of axons, reactive astrogliosis, and complement deposition. Perivascular infiltrates consisted of mixed CD4+, CD8+ and CD20+ cells with rare plasma cells. Conclusion: These pathological findings are compatible with MOG-Abs seropositive cases and reinforce the importance of testing CSF in highly suggestive cases.

Keywords: Meningitis – Myelin Oligodendrocyte Glycoprotein – cerebrospinal fluid – diagnosis – myelitis

P068

CSF proteome in multiple sclerosis subtypes related to brain lesion transcriptomes

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Introduction: Multiple sclerosis (MS) is an inflammatory neurodegenerative disease. Identification of specific molecular markers that reflect the pathology and disease course is difficult because of the dynamic complex pathogenesis. Objectives: By overlapping the brain lesion RNA signatures and CSF proteome of patients in different disease groups, we aimed to explore MS- and stage-specific molecular markers. Methods: Based on RNA next-generation sequencing of pathologically defined different lesion types in progressive (P)MS patients (n = 73) compared to white matter of non-neurological disease controls (n = 25), we created the first public interactive atlas of MS transcriptomic brain lesion maps (www.msatlas.dk). Next, we identified markers of MS subtypes: (i) Discovery proteomics compared 169 CSF from MS subtypes, other diseases (NMO spectrum and Alzheimer disease) and healthy controls. (ii) Quantitative proteomics on selected proteins were performed in 170 CSF samples. (iii) Transcripts of the proteins in MS lesions were screened by the MS Atlas. Results: 8 proteins differentiated the MS subtypes. Transcripts of 7 of these proteins were present in MS lesions e.g. FRZB in active and chronic active lesions. Volcano maps of proteins indicated the highest number of altered proteins in secondary progressive (SP) MS. 5 non-inflammatory proteins were upregulated in the MS subtypes. This profile and associated lesion spectrum highlight non-inflammatory mechanisms in differentiating CNS diseases, and the uniqueness of SPMS. Conclusion: Using multi-omics of the human CNS compartment, we were able to identify novel disease-specific markers in the CSF. Additionally, our MS Atlas can aid advancing research in MS and other neurological diseases.

Keywords: Multiple sclerosis – systems biology – CNS compartments

P069

Is involvement of visual and craniobulbar pathway in rabies encephalitis the anatomical pathway for hydrophobia?

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Introduction: Rabies, a fatal rhabdovirus infection is common in India. Occurrence of hydrophobia, a dramatic but enigmatic phenomenon associated with this disease suggests involvement of the visuo – motor pathway by the virus. Objective: To determine visual pathway involvement, we traced the rabies viral antigen distribution from retina to visual pathway in autopsied human rabies and experimental rabies in permissive adult Swiss Albino mice by immunohistochemistry. Methods: Rabies viral antigen in nine cases of rabies was mapped by immunohistochemistry using antibody to nucleoprotein of rabies virus. Only one manifested hydrophobic features, the rest had paralytic rabies and phobic spasms terminally. Permissive adult Swiss Albino mice inoculated via intramuscular/intracerebral route with virulent rabies street and lab attenuated CVS strain were evaluated similarly. Results: The retina, optic nerve, lateral geniculate body, superior colliculus and the striate cortex demonstrated rabies viral antigen. Rabies viral antigen was also found in nuclei tractus solitarius and V, VII, IX & X, XII nuclei in brainstem, the central pattern generators controlling swallowing reflex connecting via with the superior colliculus with the visual pathway to trigger swallowing reflex/phobic spasms & hydrophobia. Variable involvement of excitatory (cingulate, insular, thalamus and hypothalamus) and inhibitory centers (pontine reticular formation and periaqueductal grey) could determine occurrence of hydrophobia. Conclusion: Presence of rabies antigen was detected along the entire visual pathway to striate cortex with caudocranial gradient. Involvement of the visual pathway may trigger the stimulation of craniobulbar nuclei in brainstem modulating contraction of pharyngeal and bulbar muscles producing phobic spasms/hydrophobia.

Keywords: Rabies – hydrophobia – visual pathway – viral antigen

P070

A case of progressive multifocal leukoencephalopathy with HIV positive

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Introduction and objective: We report a case of progressive multifocal leukoencephalopathy with HIV positive. Methods: We analyzed her epidemiology, clinical manifestation, neuroimaging and laboratory test. Results: The patient was a 36-year-old right-handed man with 4 months of left upper limb numb, 2 months of left limbs weak. His brain MRI showed his right frontal and parietal cortex

ischemic, right thalamus, corona radiata and callosum ischemic infarction (subacute stage), and posterior angle of bilateral ventricles demyelination. The focus was not enhanced on brain enhancement MRI. He got treatment according to cerebral infarction and this did not relieve it. His blood test results showed decreased white cells, red cells and platelet count. His CSF test was normal. His serum NMDA, AQP4 antibody were all negative. 1 months ago, he began to have fever and could relieve after cortisone. He couldn't get better after antiviral therapy according to viral encephalitis. His bilateral vision suddenly got blind. His PET-CT scan was normal. He was not relieved after immune globulin. His nervous system examination showed his cognitive function deceased, left central facial paralysis and left limbs upper motor neuron paralysis. His bilateral light reflex was normal. However both of his eyes could not react to light. His bone marrow aspiration was normal. Finally, he got an HIV test and the result was positive. His CSF and urine of JC virus PCR test were 1,000,000 copies/mL and 2,000 copies/mL. He was diagnosed as progressive multifocal leukoencephalopathy and HIV infection. Conclusion: This case gave us a lesson and we should keep mind on HIV tests.

Keywords: Progressive multifocal leukoencephalopathy – JC virus – HIV infection

Neurodevelopment

P071

Severe telencephalic malformations in an infant carrying a de novo pathogenetic variant in *ATP1A3*

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Introduction: Nosography of cerebral malformations still relies on descriptive neuropathological features. Advances in genetics of human Central Nervous System (CNS) malformations have helped to shed some light on putative pathogenetic mechanisms. In eukaryotic cells the Na+/K+-ATPase pump is a transmembrane enzyme involved in maintenance of ion gradients across the plasma membranes, a relevant function of excitable neuronal cells. The a3 *(ATP1A3)* subunit is primarily expressed in neurons. Dominantly inherited pathogenic variants in *ATP1A3* are associated with a broad spectrum of neurological diseases, including rare childhood encephalopathies with polymicrogyria. Background: The neuropathological features are described of a 4 months old baby affected with telencephalic malformation featuring pachygyria and lissencephaly. Histologically, leptomeningeal glioneuronal heterotopia was observed corresponding to the thick, pachigyric cortex. 4-layered polymicrogyria was present in most of the remaining cortical regions. The corpus callosum was partially absent. Mild changes of both dentate and olivary nuclei were present. Myelination was unaffected. The child was carrying a novel, de novo heterozygous mutation in *ATP1A3* (Lys763del), predicting a large deletion of the protein. Conclusion: Peculiar feature of this case is the severe, selective vulnerability of the telencephalic cortex as compared to other grey structures. The patterns of cortical abnormalities are consistent with different timing of the cortical lesions, an early defect of corticogenesis possibly related to impaired neuronal migration, and a later onset polymicrogyria related to different mechanisms. The severity of the neuropathological picture may be consistent with the large deletion in *ATP1A3,* but the pattern of lesion cannot be ascribed to the defective protein exclusively.

Keywords: CNS malformation – pachygiria – polymicrogyria – ATP1A3 deletion

P071Z

Transient boundaries and compartments of developing human fetal striatum: the role of lecticans and tenascins

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Introduction: The exact relationship between the fetal and adult compartmental layout of the striatum, as well as the molecular mechanisms that rely under it, are still a matter of inconclusive debate, especially in human brain. Previous animal studies demonstrated that future striosome and matrix neurons show distinct adherent properties, could still segregate in the absence of dopamine afferents and that their early destruction does not affect early compartmental distribution of the dopaminergic innervation in the striatum. These findings suggest that the cell surface and extracellular matrix (ECM) molecules play a significant role in the formation of striatal compartments. Methods: In this study, we evaluated the expression patterns of neurocan, versican V0/1, and neurocan binding glycoprotein tenascin C in postmortal human brain from 10th postconceptional weeks (PCW) to the 3rd postnatal months. Results and conclusion: We observed early, homogenous ex-

pression of these ECM components in human fetal striatum at 11th PCW that gradually increases in complexity and begins to show clear compartimental organization at 16th PCW. Both lecticans expression ceases around 25th PCW, while the tenascin is present until 3rd postnatal month in the caudate nucleus. In midgestation period (18th – 24th PCW), all studied ECM components show prominent expression in the perimeters, a tight cell-free zone that surrounds the cell islands. Finally, the observed expression profile of the ECM components correlates well with the sequence of growth and maturation of striatal afferents, and indicates their involvement in the formation of boundaries between striatal modules.

Keywords: ECM – human fetal striatum – tenascin – neurocan – versican

P072

Systematic classification of spina bifida phenotypes

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Background: Spina bifida is an umbrella term for several phenotypes. In view of the increasing identification of spina bifida-associated genes and pathomechanisms, the exact description of spina bifida subtypes is highly important. Neuropathologic findings in cases with spina bifida are often reported with imprecise/overlapping terminology. Especially the term myelomeningocele is applied in various and divergent spina bifida subtypes. Objectives: We aimed to introduce a systematic classification of spina bifida phenotypes and to describe the spinal neuropathological findings in affected cases. Methods: We reevaluated 90 cases with spina bifida (58 prenatal; 32 postnatal), which were examined in the Institute of Neuropathology of Charité – Universitätsmedizin Berlin from 1974 to 2000. Results: Almost half of the cases (n = 40; 44%) of spina bifida cases were syndromic. The most frequently diagnosed phenotype was myeloschisis (n = 28; 31%), characterized by an open neural plate with exposed ependyma. In addition, 21 cases (23%) had myelomeningoceles, 2 cases (2%) meningoceles, and in 21 cases (23%) an unspecified aperta subtype. Spina bifida occulta was present in 7 cases (8%). 22 of 28 myeloschisis cases (79%) showed a SBA of the lower spina without additional cranial neural tube defects, only one myeloschisis case was associated with anencephaly. Conclusion: We propose a classification of spina bifida subtypes: spina bifida occulta and three spina bifida aperta subtypes (meningocele, myelomeningocele, and myeloschisis). Based on our findings and the review of literature, (i) myelomeningocele and spina bifida aperta cannot be used as synonymous terms and (ii) myeloschisis is an underreported spina bifida phenotype.

Keywords: Spina bifida – neural tube defects – myelomeningocele – myeloschisis

P073

Neuropathology of genetically defined malformations of cortical development – a systematic literature review

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Introduction: Malformations of cortical development (MCD) include a heterogeneous spectrum of clinical, imaging, molecular and histopathological entities. While the understanding of genetic causes of MCD has improved with the availability of next-generation sequencing modalities, genotype-histopathological correlations remain limited. Purpose: This is the first systematic review of molecular and neuropathological findings in patients with MCD to provide a comprehensive overview of the literature. Methods: A systematic review was performed between November 2019 and February 2020. A MEDLINE search was conducted for 132 genes previously linked to MCD in order to identify studies reporting macroscopic and/or microscopic findings in patients with a confirmed genetic cause. Results: 81 studies were included in this review reporting neuropathological features associated with pathogenic variants in 46 genes (46/132 genes, 34.8%). Four groups emerged, consisting of (1) 13 genes with well-defined histological-genotype correlations, (2) 27 genes for which neuropathological reports were limited, (3) 5 genes with conflicting neuropathological features, and (4) 87 genes for which no histological data were available. Lissencephaly and polymicrogyria were reported most frequently. Associated brain malformations were variably present, with abnormalities of the corpus callosum as the most common associated feature. Conclusion: Neuropathological data in patients with MCD with a defined genetic cause is available only for a small number of genes. As each genetic cause might lead to unique histopathological features of MCD, standardized thorough neuropathological assessment and reporting should be encouraged. Histologic features can help improve the understanding of the pathogenesis of MCD and generate hypotheses with impact on further research directions.

Keywords: Malformations of cortical development – lissencephaly – polymicrogyria – dysgyria – cobblestone malformation

P074

Cerebral abnormalities in spina bifida: a neuropathological study

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Introduction: Spina bifida (SB) is the most common neural tube defect in humans. We recently reported in a radiological study that all open SB forms are associated with cerebral pathology and with a lower cognitive profile. Objectives: The aim of this study was to systematically analyze neuropathological findings of the brain at various developmental time points in SB. Methods: 79 cases with SB aperta (SBA) and 6 cases with SB occulta (SBO) autopsied at the Charité Neuropathology from 1974 to 2000 were re-evaluated retrospectively. For this, case files and spinal cord as well as brain sections were studied. Results: While no brain malformations were detected in SBO cases, 95% of SBA cases had brain malformations. Main brain anomalies identified were hydrocephalus (71%), heterotopia (41%), Chiari II malformation (36%), other cerebellar anomalies than Chiari II (36%), gyrification defects (33%), and ependymal denudation (29%). Hydrocephalus was detected early, already at gestational week 17, and was present in over half of the prenatal cases (n = 27). Chiari II malformation and loss of ependymal lining were highly associated with hydrocephalus. Conclusion: We confirm using neuropathologic methods a wide range of brain malformations in most SBA but none in SBO cases. In addition to our previous radiologic study, we now demonstrate the high prevalence of cerebral malformations and cerebellar heterotopias in SBA. The early detection of hydrocephalus and Chiari II malformation in fetuses raises the question whether these arise parallel rather than in strict temporal sequence.

Keywords: Spina bifida – Chiari II malformation – hydrocephalus – myelomeningocele

P075

Limited anatomical and microscopic findings in a case of Wolf-Hirschhorn Syndrome highlight the spectrum of disease

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Introduction: Wolf-Hirschhorn (4p-) syndrome (WHS) is one of the most common microdeletion syndromes with an incidence of 1 : 50,000 births. Yet, while the craniofacial features and other congenital anomalies are well-appreciated, CNS findings are less well-described. We present a case of confirmed WHS with multiple external (low set ears, bilateral orbital creases, contractures, talipes equinovarus, ambiguous genitalia, urethral agenesis), skeletal (cervical rib, supernumerary thoracic vertebrae, segmented sacral vertebrae) and internal (bilateral renal agenesis, Meckel's diverticulum, intestinal malrotation, rectal atresia, pulmonary hypoplasia, absent innominate vein, retroesophageal right subclavian vein) anomalies. Material and methods: The brain from a 20-week fetus was routinely fixed in 10% formalin, dissected, embedded in paraffin blocks and stained with H&E. SNP microarray was performed on villous tissue. Results: A mosaic terminal deletion in approximately 35 to 40% of cells was present involving chromosome 4 within 4p16.13p14. The deletion encompassed, but was much larger than, the region that is associated with WHS. Neuroautopsy showed a normal sized brain without many of the previously described gross CNS anomalies such as defects of gyration or heterotopias. Corpus callosum agenesis was identified and microscopic findings included focally microcystic white matter and atrophy of neurons in the cerebellar dentate nucleus. Conclusion: Various deletion sizes occur in the short arm of chromosome 4 and demonstrate a relatively wide phenotypic spectrum of neuropathological findings in Wolf-Hirschhorn syndrome.

Keywords: Wolf-Hirschhorn syndrome – WHS – microdeletion syndrome

P076

Temporal changes in the brain in neonatal hydrocephalic mice: structural and neurobehavioral findings

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Introduction: In hydrocephalus, the circulation of cerebrospinal fluid (CSF) is altered leading to its accumulation in the ventricles and subarachnoid space. The impact of this disease on neurobehaviour and the structure of the cellular organelles of pyramidal neurons and their synapses in the neonatal hydrocephalic mouse brain overtime are not fully understood. Objective: To evaluate the pyramidal neurons and their synapses in the sensorimotor cortex of neonatal hydrocephalic mice. Methods: Hydrocephalus was induced in day-old mice. The pups were tested for reflex development prior to sacrifice on postnatal days 7, 14, 21. Cortical thickness, neuronal density and pyknotic cell count in the sensorimotor cortex were evaluated. Ultrathin stained sections were evaluated and quantitative analysis of the synapses done. Results: Surface righting reflex and cliff avoidance activities were significantly impaired in hydrocephalic pups. The cortical thickness of the hydrocephalic mice was significantly reduced on PND 7 compared to controls. Compared with age-matched controls ($129.60 \pm 3.72 \times 10^{-6}$ μm²; $230.0 \pm 44.1 \times 10^{-6}$ μm²), the neuronal density of the sensorimotor cortex in hydrocephalic mice was significantly increased on PND 14 ($157.70 \pm 21.88 \times 10^{-6}$ μm²) and PND 21 ($373.20 \pm 21.54 \times 10^{-6}$ μm²). The TEM of the neuropil of the hydrocephalic mice showed loss of structural integrity of cellular organelles. The synaptic densities (per μm² × 10^{-5}) of the hydrocephalic mice were significantly lower (188.0 ± 22.67; 120.0 ± 21.68; 72.0 ± 0.66) than their age-matched controls (336.0 ± 37.09; 486.0 ± 18.60; 600.0 ± 17.61) on days 7, 14, and 21 respectively. Con-

clusion: The findings seen in the neuronal population of the hydrocephalic mice may provide supportive data for the structural basis of the neurological disabilities associated with neonatal hydrocephalus.

Keywords: Transmission electron microscopy – neonatal hydrocephalus – sensorimotor cortex – pyramidal neurons – neurobehavior

P077

Neuropathological findings in a patient with Fowler syndrome surviving into adulthood: an autopsy report

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Introduction and objective: Proliferative Vasculopathy and Hydranencephaly-Hydrocephaly syndrome (PVHH), also known as Fowler syndrome, is a rare autosomal recessive disorder of brain angiogenesis due to *FLVCR2* mutations. Most described cases were prenatally fatal. Only 6/72 described cases survived beyond birth. We describe neuropathological findings in one of three siblings who survived into adulthood. Methods: An autopsy was performed on an 18-year old woman, one of three siblings in whom whole exome sequencing had revealed compound heterozygous variants in *FLVCR2*. The brain was examined with routine neuropathological methods. Results: On gross examination the brain weighed 440 gram. Ventriculomegaly was observed more in the occipital lobe than in the frontal lobe. The corpus callosum was thinned. Microscopic examination showed multiple microcalcifications in the gyral white matter, deep nuclei and the brain stem. Tissue loss and gliosis were seen around these calcifications. There was spongiosis, gliosis and thinning of the cerebral cortex due to neuronal loss. Glomerular vascular malformations were not present. Conclusion: Neuropathological findings in an adult case of Fowler syndrome are compatible with prenatal tissue destruction as can be seen due to focal ischemia in the white matter and the brain stem. The intracortical neuronal loss with spongiosis and gliosis are reminiscent to the cortical lesions seen in mitochondrial diseases, such as Alpers syndrome. However, the clinical course and the genetic basis in Alpers syndrome are different from the cases described here.

Keywords: Fowler syndrome – FLVCR2 – brain autopsy

Epilepsy

P078

Loss of capillary low-density lipoprotein receptor-related protein 1 expression in the hippocampus of temporal lobe epilepsy and Alzheimer's disease patients

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Background: Recently it has been reported that temporal lobe epilepsy (TLE) patients have increased brain amyloid beta (Aβ) expression, a neuropathological hallmark of Alzheimer's Disease (AD). Aβ accumulation might also explain the increased risk of developing epilepsy in AD. Objective: Since the low-density lipoprotein receptor-related protein 1 (LRP1) may be involved in Aβ clearance from the brain, we investigated LRP1 expression in the hippocampus of TLE and AD patients. Methods: Using immunohistochemistry, we studied the expression and cellular localization of LRP1 on hippocampal sections of autopsy controls (n = 20), AD patients (n = 12) and resected hippocampal tissue of age-matched TLE patients with hippocampal sclerosis (TLE-HS; n = 14). Results: We demonstrated strong LRP1 expression at the abluminal side of brain capillaries in control specimens. Optical density

analysis showed lower LRP1 expression in capillaries ($p < 0.05$) and higher expression in astrocytes ($p < 0.05$) in the dentate gyrus, CA1 and CA3 region of the hippocampus from AD and TLE-HS patients as compared to controls. Conclusion: Our results suggest that downregulation of LRP1 in brain endothelial cells could be involved in increased perivascular Aβ accumulation. In future studies we will investigate whether restoration of endothelial LRP1 expression can prevent or attenuate the development of epilepsy and/or accumulation of Aβ.

Keywords: Low-density lipoprotein receptor-related protein 1 – temporal lobe epilepsy – Alzheimer's disease

P079

Iron accumulation and dysregulated iron metabolism after status epilepticus and temporal lobe epilepsy

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Introduction: Neuronal dysfunction due to iron accumulation in conjunction with reactive oxygen species (ROS) could represent an important component of the epileptogenic process. However, to date alterations in iron metabolism in the epileptogenic brain have not been addressed in detail. Methods: Iron-related neuropathology and antioxidant metabolic processes were investigated in resected brain tissue from patients with temporal lobe epilepsy and hippocampal sclerosis (TLE-HS), post mortem brain tissue from patients who died after status epilepticus (SE) as well as brain tissue from the electrically-induced SE rat model of TLE. Finally, the effects of iron overload were studied in vitro using an acute mouse hippocampal slice preparation and cultured human fetal astrocytes. Results: While iron accumulating neurons had a pyknotic morphology, astrocytes appeared to acquire iron-sequestering capacity as indicated by prominent ferritin expression and iron retention in the hippocampus of patients with SE or TLE. Post-SE rats had consistently higher hippocampal iron levels during the acute and chronic phase (when spontaneous recurrent seizures are evident). In vitro, in acute slices that were exposed to iron, neurons readily took up iron, which was exacerbated by induced epileptiform activity. Fetal astrocyte cultures challenged with iron and ROS upregulated their anti-oxidant and iron capacity, but simultaneously developed a pro-inflammatory phenotype upon chronic exposure. Conclusion: These data suggest that seizure-mediated, chronic neuronal iron uptake might play a role in neuronal dysfunction/loss in TLE-HS. On the other hand, astrocytes sequester iron, specifically in chronic epilepsy. This function might transform astrocytes into a highly resistant, pro-inflammatory phenotype potentially contributing to pro-epileptogenic neuroinflammation.

Keywords: Temporal lobe epilepsy – iron – astrocytes – oxidative stress

P080/SY 9.6

The antiseizure and antiepileptogenic effect of matrix metalloproteinase inhibitor IPR-179 and its potential mechanisms of action

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Introduction: Matrix metalloproteinases (MMPs) are extracellular endopeptidases acting as modulators of neuroinflammation and blood-brain barrier (BBB) integrity. Epileptogenesis is associated with increased expression of MMPs which therefore may represent potential therapeutic targets. Objectives: In this study, we further investigate MMPs in epilepsy and examine the therapeutic potential of the MMP2/MMP9 inhibitor IPR-179. Methods: Using qPCR and immunohistochemistry, we studied the expression of MMPs and their endogenous inhibitors (TIMPs) in temporal lobe epilepsy (TLE) patients and in a rat TLE model. Furthermore, we tested IPR-179 in the rapid-kindling rat model and the intrahippocampal kainic acid mouse model, followed by ex vivo brain examination. Human fetal astrocytes and an in vitro BBB model containing both astrocytes and endothelial cells were challenged and treated with IPR-179 to evaluate the potential mechanisms of action. Results: In both human and experimental epilepsy, MMP and TIMP expression were dysregulated in the hippocampus compared to controls. IPR-179 treatment reduced seizure severity in the rapid-kindling model and reduced the number of spontaneous seizures in the kainic acid model

(during and up to 7 weeks after delivery) without side effects while improving cognitive behavior. Preliminary data show that MMP inhibition by IPR-179 can attenuate a proinflammatory astrocytic response as well as rescue induced BBB permeability. Conclusion: Increased MMP expression is a prominent hallmark of the human epileptogenic brain and the MMP inhibitor IPR-179 exhibits antiseizure and antiepileptogenic effects in rodent epilepsy models potentially via attenuation of neuroinflammation and BBB disruption. As IPR-179 ameliorates seizure-induced cognitive decline, it deserves further investigation in clinical trials.

Keywords: Matrix metalloproteinase – epilepsy – epileptogenesis – blood-brain barrier – therapy

P081/SY 9.4

Mimicking white matter pathology in a 3D-nanofiber cell culture system derived from children with drug-resistant epilepsies

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Background: Focal cortical dysplasia type 2B (FCD2B) and the histologically indistinguishable cortical tuber of tuberous sclerosis complex patients (TSC) are often associated with early-onset pharmaco-resistant epilepsy in children. One shared major pathologic aspect is the aberrant myelination of the white matter. We recently reported impaired oligodendroglial turnover with depleted myelin and oligodendroglia in FCD2B and TSC patients. Objectives: Data of the dynamics of oligodendrocyte biology and myelin formation are scarce. Therefore, we characterized the pathophysiology of abnormal myelin formation in a cell culture model of pediatric epilepsy surgery patients. Methods: We analyzed primary mixed glial cell cultures derived from epilepsy surgery specimens of one TSC and seven FCD2B patients grown on polycaprolactone fiber matrices. Samples of unaffected white matter of three age-matched patients with Mild Malformations of Cortical Development (MMCD) served as controls. Immunofluorescence, western blot, fiber metrics and electron microscopy were applied to characterize oligodendrocyte and myelination dynamics. Results: Our preliminary results suggest that cells derived from TSC and FCD2B surgery specimens cultured on a three-dimensional nanofiber scaffold show altered myelination capacity compared with MMCD cell cultures. We demonstrate higher amounts of oligodendroglial precursor cells but an overall lower content of mature oligodendroglia and myelinated fibers. In our culture model, we were able to observe reduced regenerative capacity of oligodendroglial cells in TSC and FCDIIB when compared to MMCD. Conclusion: Our study showed for the first time a more functional proof of oligodendrocytes affected by the malformative process per se rather than being inactive bystanders.

Keywords: Focal cortical dysplasia 2B – tuberous sclerosis complex – oligodendrocyte – nanofibers – myelination

P082/SY 9.5

DNA methylation-based classification of malformations of cortical development

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Background: Accurate histopathological diagnosis is crucial for optimal management of patients with epilepsy. For the spectrum of malformations of cortical development (MCD), standardization of the diagnostic process has been shown to be challenging. Some molecular grouping has been introduced including genetic marks, but only for selected entities. Diagnostic discordance and uncertainty may confound the assignment of genetic variants to disease entities and compromise decision-making in clinical practice. Other objective molecular markers should be considered. Objectives: It has been convincingly shown that particularly DNA methylation profiling is highly robust and reproducible even from small samples and formalin-fixed paraffin-embedded (FFPE) tissue, and such profiles have been widely used to classify CNS tumors. In line with these findings, we recently provided the first evidence that epilepsy-associated structural brain lesions can be classified based on DNA methylation. Methods: Here we analyzed genome-wide DNA methylation patterns of almost 300 MCD patients and no seizure controls using Illumina 850K arrays to identify distinct methylation classes of MCD applying three different approaches, i.e., pairwise comparison, machine learning, and deep learning. Results: Our data clearly show the suitability of DNA methylation-based MCD classification across all major histopathological entities amenable to epilepsy surgery and age groups, and its potential application in a routine diagnostic setting. Conclusion: The availability of this method may have a substantial impact on diagnostic precision compared to standard methods. Our results provide a blueprint for the generation of

machine-learning-based disease classifiers across other epilepsy-associated structural brain lesions, with the potential to fundamentally transform epilepsy neuropathology.

Keywords: Epilepsy – MCD – DNA methylation – classification

P083

Increased expression of complement components in tuberous sclerosis complex and focal cortical dysplasia 2b brain lesions

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Background: Increasing evidence supports the contribution of inflammatory mechanisms to the neurological manifestations of epileptogenic developmental pathologies linked to mTOR pathway dysregulation (mTORopathies), such as tuberous sclerosis complex (TSC) and focal cortical dysplasia (FCD). Using high-throughput RNA sequencing we previously observed elevated expression of genes encoding components of the complement system in TSC brain tissue. Objectives: The aim of this study is to investigate the expression pattern and cellular distribution of complement factors C1q and C3 in resected cortical tissue of clinically well-characterized patients with TSC or FCD2b. Method: Quantitative real-time PCR and immunohistochemistry was performed on TSC (n = 20 and 27, respectively), FCD2b (n = 8 and 32, respectively) and autopsy control cortex (n = 20). Furthermore, immunofluorescent characterization was performed with astroglial, microglial and neuronal markers. Results: C1q and C3 mRNA and protein expression was upregulated in TSC and FCD samples compared to controls. Moreover, double staining confirmed co-localization of complement factors with glial and neuronal cells in lesional and perilesional areas. Conclusion: Our results demonstrate that prominent activation of the complement pathway represents a pathological hallmark of TSC and FCD2b, suggesting that this may contribute to the pathology that underlies these epileptogenic developmental diseases. Further functional experiments should reveal the biological relevance of the complement pathway in relation to mTOR pathway deregulation in mTORopathies and as a potential therapeutic target.

Keywords: TSC – FCD – complement – inflammation

P083Z

The histoarchitectonic glycosylation pattern of diffused and perineuronal condensed extracellular matrixin hippocampal sclerosis

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Introduction: Hippocampal sclerosis (HS) is the commonest histopathological finding in patients with drug-resistant mesial temporal lobe epilepsy (MTLE). The type 1 HS (ILAE type 1), with severe pyramidal neurons (PyN) loss in CA1 and CA4 regions as hallmark, and parvalbumin interneurons number and connectivity, depletion is most fre-

quent. Currently, extracellular matrix (ECM) is recognized as an important regulator of excitability and synaptic plasticity, especially its condensed peri-cellular form, perineuronal nets (PNN). The different ECM components and PNN are suspected to have role in epileptogenesis in experimental rodent models. Aim and methods: Aim of this retrospective study is to reveal the significance of PNN and other characteristics of ECM by correlating them to cellular (NeuN, GFAP, PV), molecular (WFA), histoarchitectonical and clinical findings from 65 patents surgically treated due to pharmacoresistant MTLE caused by HS. Results: In addition to previously shown features of HS1, as reduced number and impoverish morphology of Py and PV neurons, we found significant negative correlation of PV-neurons in CA4 and GD with number of antiepileptic drugs used at the same time during period of one year before surgery. We also found a change in the distribution of the specific glycosylation pattern recognized by WFA, presented as partially degraded PNNs, but concomitantly highly WFA positive glycan component of the dispersed ECM in CA4. The PNN are reduced around pyramidal neurons in all regions (CA4-CA1) as well as around PV-immunoreactive interneurons. Conclusion: PNN reduction and change of the glycosylation profile of the neuropil in the HS1-MTLE possibly contribute to the pathophysiology of drug-resistant epilepsy, and might be possible ground for novel therapies.

Keywords: MTLE – perineuronal nets – drug-resistant epilepsy

P084

Histopathological findings in brain tissue of patients with pharmacoresistant epilepsy

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Introduction: Pharmacoresistant epilepsy represents 30 – 40% of cases with intractable epilepsy in children, and up to 20% of adult cases. Various cerebral lesions can lead to drug-resistant focal epilepsy. They are medically refractory but the complete surgical resection correlates with good outcome. Objectives: The purpose of our study is to identify the histopathological findings, which can lead to pharmacoresistant epilepsy, and to evaluate the relationships between histology and postoperative seizure control. Methods: Our study included 205 histologically proven cases, diagnosed during the period from 2006 to 2019 in the Epilepsy Surgery Center of University Hospital "Saint Ivan Rilski", Sofia, Bulgaria. Brain tissue materials were embedded in paraffin using routine histological practice. Tissue sections were deparaffinized and stained with haematoxylin and eosin. Further histo- and immunohistochemical examinations were performed. Results: 205 patients with drug-resistant epilepsy underwent neurosurgery and were pathomorphologically examined between 2006 and 2019. The most common pathomorphological findings were: focal cortical dysplasia (n = 95), hippocampal sclerosis (n = 24), dysembryoplastic neuroepithelial tumor (n = 21), ganglioglioma (n = 18), glial scars/gliosis (n = 16), vascular lesions (n = 14), encephalitis (n = 6), pilocytic astrocytoma (n = 3), pleomorphic xanthoastrocytoma (n = 2), tuberous sclerosis (n = 2), epidermoid cyst (n = 2), hypothalamic hamartoma (n = 1), papillary glioneuronal tumor (n = 1). Conclusion: The most frequent causes of pharmacoresistant epilepsy are malformations of cortical development, hippocampal sclerosis and glioneuronal tumors. The targeted magnetic resonance imaging in detecting epileptogenic lesions, complete surgical resection and accurate histological diagnosis allow effective postoperative seizure control in cases with drug-resistant epilepsy.

Keywords: Pharmacoresistant epilepsy – histopathological findings – focal cortical dysplasia

P085

Ectopic neurons in deep white matter of the temporal lobe: histopathological assessment in epilepsy – a case-controlled retrospective study

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Introduction: Ectopic neurons in the white matter of the temporal lobe have been widely reported in patients with epilepsy. Determining their pathological significance has been challenging due to variable numbers noted in previous research amongst normal and epilepsy cases. Furthermore, cases examined with underlying neurological disorders may also be contributing to white matter ectopic neurons. Analysis of purely epilepsy cases versus neurologically normal controls is limited in considering threshold values of white matter ectopic neurons as abnormal. Objectives: To investigate the pathological significance and threshold of ectopic white matter neurons in patients with epilepsy. Methods: We compare 10 cases of sudden unexpected death in epilepsy (SUDEP) with 13 controls in the temporal lobe of post-mortem brains. Samples stained with hematoxylin and eosin (H&E) were examined at × 40 magnification, known as a high-power field (HPF). The density of ectopic neurons was taken as the number of white matter neurons per 10 HPF's, and mean neuronal density was measured. Results: A mean of 3.00 neurons per 10 HPF was found in SUDEP cases, compared to a mean of 0.62 neurons per 10 HPF in control subjects. The distribution of the two cohorts differed significantly ($U = 99.00$, $p = 0.036$). A threshold of > 4 ectopic neurons per 10 HPF is determined as pathological. Conclusion: A greater mean density of ectopic white matter neurons in epilepsy cases compared to controls suggests a clinical-pathological association. We propose a threshold of greater > 4 ectopic neurons per 10 HPF as a pathological finding in epilepsy.

Keywords: Epilepsy – temporal lobe – ectopic neurons – white matter

P086

Cortical neuronal hypertrophy and MTOR pathway activation in autonomic brain regions in SUDEP

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Background: Dysfunctional connectivity and pre-existing structural abnormalities of central autonomic network regions (CAN) have been shown on MRI in SUDEP. In a previous post-mortem (PM) study we reported increased microglia in the superior temporal gyrus (STG) in SUDEP. Objectives: Our aim was to quantify pS6 (MTOR pathway activation) and neuronal c-Fos (neuronal activation marker) in CAN regions in SUDEP compared to control groups. Methods: In a series of 58 PM cases, including SUDEP, epilepsy controls (EPC) and non-epilepsy controls (NEC) we quantified pS6-240, pS6-235, and c-Fos neuronal labelling (neuronal densities (ND) and percentage positive neurones (PP)) in the STG, anterior cingulate, insular, fronto-basal and pulvinar regions using quantitative immunohistochemistry with whole slide image analysis. Results: Neuronal and glial pS6 and c-Fos labelling was variable between cases and brain regions. Cortical neuronal hypertrophy in the STG was observed in some SUDEP cases and pS6-240 highlighted hypertrophic neurones. pS6-235 highlighted neuronal intranuclear inclusions and significantly more pS6-235-positive neurones were present in the STG than other regions in SUDEP ($p < 0.05$). Higher c-Fos ND in both the insular and STG than pulvinar regions was noted, but with greater differences in the SUDEP group ($p < 0.001$). Higher c-Fos PP and ND was present in the STG in SUDEP compared to NEC ($p \leq 0.05$). Conclusion: Neuronal labelling for pS6-235, pS6-240 and c-Fos may highlight cortical regions with increased seizure activity in the period prior to death which could be relevant to mechanisms in seizure-related deaths. Relatively increased STG neuronal expression may represent a specific signature in SUDEP.

Keywords: Sudep – Mtor – Epilepsy – C-Fos

Tumors – gliomas

P087

A case report of a novel NTRK-gene fusion in pleomorphic xanthoastrocytoma

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Introduction and objective: Pleomorphic xanthoastrocytoma (PXA) is a central nervous system tumor accounting for < 1% of all astrocytomas. It is classified as WHO grade II and is associated with a 5-year survival of > 75%. In this case report we describe a new NTRK fusion, which opens a new perspective for treatment. Methods: A 6-year old child presented with recurrent episodes of absence seizures. MRI showed a cystic process on the left mesiotemporal side. Pathological examination was performed. Results: Histopathological examination revealed a pleomorphic tumor. Regions with rather spindled cells were intermixed with regions showing more epithelioid or bizarre cells. The nuclei were irregular and had striking nucleoli. Some larger cells showed foamy cytoplasm. Immunohistochemical examination was positive with GFAP. CD34 was positive in some epithelioid cells. Synaptophysin was focally positive. Both immunohistochemistry and next generation sequencing revealed a NTRK-gene fusion. Discussion: PXA can be divided into BRAF-mutated and BRAF-wild type tumors. BRAF mutation is associated with a survival benefit. Treatment with BRAF inhibition has been associated with marked radiological responses in BRAF-mutated PXA. Recently, another category of treatable molecular alterations has been described in cancer, namely fusions including the NTRK-gene family. Larotrectinib and entrectinib are used for treatment of these fusions. In BRAF-wild type cases, our findings suggest that at least (N)TRK status should be evaluated when considering neo-adjuvant therapy or for progressive or recurrent disorder. Conclusion: We present the first case of a *TPM4-NTRK2*-fused PXA, stressing the need for NTRK-testing in BRAF-wild-type PXA cases.

Keywords: Pleomorphic xanthoastrocytoma – NTRK-fusion – molecular

P088

Unexpected presentation of an IDH-mutant glioblastoma at autopsy

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Background: A 32-year-old man died suddenly and unexpectedly following a short history of headache

and nausea and following extensive use of nitrous oxide canisters. Objectives: No cause of death was evident during general post mortem examination and a neuropathology consult was arranged to investigate the cause of death with potential focus on the effect of nitrous oxide use on the brain. Methods: Fixed brain examination followed by extensive sampling for histology with routine Hematoxylin & Eosin staining as well as immunohistochemistry including markers for IDH1 R132H, ATRX, p53, and MIB-1. Consent for teaching and research is in place. Results: Fixed brain examination revealed increased brain weight and on slicing an ill-defined extensive lesion with cystic change vaguely centered on the anterior corpus callosum was identified. On histology, extensive infiltration by a glioblastoma, IDH-mutant (WHO grade IV) was confirmed. It carried the IDH1 R132H mutation and ATRX was lost. Macroscopic images and the extent of microscopic infiltration will be illustrated and discussed. Conclusion: This is an unusual presentation of a glioblastoma in a young adult, which, had the brain not been sent for formal neuropathological examination, might have been ascribed to excessive nitrous oxide use on balance of probabilities. The brain was extensively infiltrated by tumor and this case serves as a reminder that primary brain tumors are still being missed during life and a low threshold for neuropathological post-mortem examination continues to be important, particularly in the context of declining post-mortem numbers and limited resources.

Keywords: Autopsy – nitrous oxide – glioblastoma

P089

PAX2 immunoexpression in adult glioblastoma

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Background and objective: There is still very little information available about PAX2 and adult glioblastomas (GB). We aim to study the PAX2 immunoexpression in human GBs, studying the relationship between PAX2 immunoexpression and some clinical data with prognostic significance (IDH1, MGMT, age at diagnosis) as well as without prognostic significance (tumor localization and GB subtype). We also analyzed the relevance of PAX2 and immunohistochemical expression of stem cell markers (SOX2, CD44, Nestin, OLIG2) and other proteins as Ki67, P53, YLK40, EGFR, P16, and PTEN. Methodology: In sum, 280 GB cases treated with the Stupp schema were studied both, at the morphological and molecular levels. TMA were built and immunohistochemistry study was performed. Molecular study was done to know the MGMT promoter methylation status. GB subtypes were established based on RNA-seq studies. Statistical studies were based on the Rho Spearman Correlation, Ji2 test, Kaplan–Meier Test, and the Long-Ramp test. Results and conclusion: Data were available in 263 cases. PAX2 was negative in 89 cases (33.8%) and positive in 174 cases (66.2%). PAX2 showed a diffuse immunostaining pattern. A statistically significant relationship was obtained with age ($p = 0.018$), tumor location ($p = 0.004$), and nestin immunoexpression ($p = 0.034$). Our series determined that PAX2 immunoexpression is not related to glioblastoma subtypes and has no prognostic significance. We conclude that PAX2 immunohistochemical expression is not useful for the clinical management of glioblastoma.

Keywords: Glioblastoma – Pax2 – immunohistochemistry

P090

The evaluation of palisading necrosis, vascular endothelial proliferation morphology using digital pathology systems and their relations with prognosis

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Introduction: Glioblastoma (GBM) is the highest grade malignant astrocytic tumor and has a poor prognosis. It is a highly cellular tumor and typically has palisading necrosis (PA) and vascular endothelial proliferation (VEP) different from low grade glial tumors. It is divided into two groups as IDH mutant type and IDH wild type in Word Health Organization classification of tumors of central nervous system 2016. IDH wild types are known to have a worse prognosis. The aim of the study is to determine the relation between histopathological features such as cellularity, PA and VEP with prognosis in these tumors. Methods: The study includes 33 cases that diagnosed IDH wild type glioblastoma. Patients consist of 12 women and 21 men. Median survival of patients is 11 months. 2 year survival rate of patients is 29% and 5 year survival rate is 4%. Total tumor areas (mm^2) (TA), palisading necrosis areas (mm^2) (PNA), vascular endothelial proliferation areas (mm^2) (VEPA) and number of cells in 0.5 mm^2 area were calculated for each case using digital pathology systems. The percentage of PNA and VEPA relative to tumor areas was calculated. VEPAs were calculated separately in two groups as multilayered (mVEP) and glomeruloid (gVEP). Conclusion: Significant cut-off value was not found in ROC analysis for between survival and variables. Variables grouped by median values and Kaplan-Meier method was applied. Each parameter were statistically insignificant. However, patients with more PNA and gVEPA

had longer median survival times. Significant results could be obtained with a higher number of patients for these parameters.

Keywords: Glioblastoma – palisading necrosis – vascular endothelial proliferation

P091

Total DNA methylation and 8-oxo-deoxyguanosine as markers of DNA damage and tumor malignancy in gliomas

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Introduction: DNA methylation (5-methylcytosine, m^5C) is a central epigenetic mechanism controlling cellular processes. Increased production of reactive oxygen species (ROS) has broad influence on cancer development, and resistance to treatment. Many ROS-derived DNA modifications can be used to monitor pathologic processes. m^5C along with other basic components of DNA are the targets for ROS what results in the appearance of modified nucleic acid bases. Many studies also showed that 8-oxo-deoxyguanosine (8-oxo-dG), oxidative DNA damage marker, is extensively present in cancer cells. Objective: The aim of our study was to provide an insight into the relation of DNA methylation and oxidative DNA damage with glioma malignancy. Methods: We analyzed total DNA contents of 5-methylcytosine (^{32}P-postlabeling method) and 8-oxo-deoxyguanosine (electrochemical detection) in brain glioma tissues and peripheral blood samples. Results: The level of m^5C was decreasing as the malignancy was increasing. We also observed a stepwise increase of 8-oxo-dG contents in DNA from brain glioma tissue with increasing tumor grade. Low levels of total DNA methylation were concomitant with high levels of 8-oxo-dG and in higher grade gliomas. The results obtained from peripheral blood samples DNA were concordant with that relation. Conclusion: We've shown a direct correlation between the oxidative DNA damage (monitored by 8-oxo-dG) and epigenetic regulation (through total m^5C), and their relation with tumor malignancy. That is a step further than nowadays existing classification systems, and holds a potential for better patient stratification in terms of treatment planning and prognosis.

Keywords: Glioma – DNA methylation – oxidative stress – 8-oxo-deoxyguanosine – malignancy

P092

Postoperative prognostic nomogram for adult grade II/III astrocytoma in Chinese Han people

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Background: Glioma is the most common type of brain tumor in adults. A nomogram is a graphical depiction of a prediction model that can assess the overall probability of a specific outcome for any individual patient. Objectives: This study was aimed to develop and independently validate a nomogram to predict overall survival (OS) for adult Chinese Han patients with newly diagnosed adult grade II/III astrocytoma after surgery, using data from two hospitals. Methods: This study retrospectively examined 722 patients (Grades II – III) with astrocytoma. We used 472 patients from Qilu Hospital to construct a nomogram based on Cox proportional hazards model. The nomogram was internally validated using 1,000-bootstrap resampling replicates and individually predicted 1-, 3-, and 5-years survival probabilities in an independent sample of 250 patients from Linyi People's Hospital. The nomogram's performance was evaluated with discrimination (concordance index) and calibration. Results: OS was negatively associated with histopathology, age at diagnosis, subtotal resection, multiple tumor, lower Karnofsky performance status score and midline tumor. Internal validation with bootstrap resampling and external validation showed good discrimination (C-index for

5-year survival is 0.733 for internal validation and 0.730 for external validation). The calibration curve indicated good agreement between prediction and actual observation. Conclusion: This is the first nomogram that integrates common clinicopathologic factors to provide an individual probabilistic prognosis prediction for Chinese Han patients with astrocytoma (Grades II – III). This model may serve as an easy-to-use tool to advise patients and establish optimizing surveillance approaches after surgery.

Lijie Wang and Jingtao Wang contributed equally to this article.

Keywords: Astrocytoma – Cox regression – nomogram – survival – predictive accuracy

P093

Genetic and environmental determinants of *O*^{6}-methylguanine DNA-methyltransferase (*MGMT*) gene methylation: a 10-year longitudinal study of Danish twins

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Background: Epigenetic inactivation of *MGMT* is associated with increased sensitivity to alkylating chemotherapeutic agents in glioblastoma patients. Objectives: Making use of the longitudinal twin design, we aimed, for the first time, to estimate the genetic contributions to *MGMT* methylation in a Danish twin cohort. Methods: DNA-methylation from whole blood (18 monozygotic and 25 dizygotic twin pairs) repeated 10 years apart from the Longitudinal Study of Aging Danish Twins were used to search for genetic and environmental contributions to DNA-methylation at 170 CpG sites across the *MGMT* gene. Both univariate and bivariate twin models were applied. The intraclass correlations, performed on cross-sectional data (246 MZ twin pairs) from an independent study population, the Middle-Aged Danish Twins, were used to assess the genetic influence at each CpG site of *MGMT* for replication. Results: There were 6 significant CpG sites, located at the gene body region, that overlapped among the two waves ($h^2 > 0.5$), of which 5 remained significant in the bivariate twin model, which was applied to both waves. Within MZ pair correlation in these six CpGs from MADT demarks the top level of genetic influence. There were 11 CpGs constantly having substantial common environmental components over the 10 years. Conclusion: We have identified 6 CpGs linked to the *MGMT* gene with strong and persistent genetic control based on their DNA methylation levels. The genetic basis of *MGMT* gene methylation may explain individual differences in glioblastoma treatment response and most importantly, provide references for mapping the methylation Quantitative Trait Loci underlying the genetic regulation. Comments to Reviewer: This paper uses classical twin models to detect meQTLs for MGMT genes whose expression activity is highly related to glioblastoma treatment response. The results were replicated in an independent twin cohort. Our paper points out the importance of genetic control over MGMT gene activity through allele-specific methylation (ASM) in the gene body, a novel finding that could help with explaining individual difference in glioblastoma treatment response while suggesting that genetic control on MGMT methylation in gene body as an alternative regulatory mechanism in addition to the well-studied promoter methylation. We sincerely hope that our findings can be of interest to readers.

Lijie Wang and Afsaneh Mohammadnejad contributed equally to this work.

Keywords: MGMT – DNA methylation – CpG site – twin models – heritability – glioma

P094

The impact of time from surgery to radiochemotherapy on overall survival in patients with newly diagnosed glioblastoma

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Introduction: Newly diagnosed glioblastoma is initially treated with surgery, and tumor tissue samples are used for histological and molecular diagnosis. Concomitant chemoradiotherapy awaits pathological determination. Recent literature found no significant benefit of early initiation of concomitant chemoradiation and adjuvant chemotherapy in patients with glioblastoma (WHO grade IV). Objectives: Examine the impact of delay in concomitant radiochemotherapy on overall survival in patients with newly diagnosed glioblastomas. Methods: Patient data from electronic medical records on patients who received surgical treatment for newly diagnosed glioblastoma at Odense University Hospital from 1 of January 2013 till 31 of December 2018 are extracted, and outcomes are

evaluated as single- and multivariate with hazard ratio. Results: Preliminary data suggest a negative impact on survival by delaying concomitant chemoradiotherapy in patients with glioblastoma relative to surgery. Data will be available for presentation at the conference. Conclusion: Preliminary data suggest a reduced overall survival in patients where concomitant chemoradiotherapy is delayed after surgery.

Keywords: Glioblastoma – timing – radiotherapy – overall survival

P095/SY 11.5

Subependymoma of the posterior fossa may progress to ependymoma: role of TERT mutation, loss of chromosome 6 and methylome alterations

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Background: Subependymomas are benign tumors characteristically encountered in the posterior fossa of adults that show distinct epigenetic profiles of the molecular group "subependymoma, posterior fossa" (PFSE). In contrast, most posterior fossa ependymomas exhibit a more aggressive biological behavior and are allocated to the molecular subgroups PFA or PFB. Objectives: The notion that a subset of ependymomas is classified as PFSE prompted us to characterize histopathological, molecular and clinical features of PFSE tumors. Methods: DNA methylation profiling was performed in a series of 50 PFSE tumors. Targeted next-generation sequencing was performed in 12 samples and TERT promoter sequencing was performed in all samples. Follow-up information was available for 49 patients. Results: On histopathology, tumors comprised 14 subependymomas, 12 ependymomas and 24 mixed ependymoma-subependymomas. Mixed ependymoma-subependymoma tumors varied in their extent of ependymoma differentiation (2 – 95%) but consistently exhibited global epigenetic profiles of the PFSE group. Methylome analysis of microdissected tumor components revealed CpG signatures in mixed tumors that coalesce with their pure counterparts. Loss of chr6 (20/50 cases) as well as TERT promoter mutations (21/50 cases) were frequent events enriched in tumors with pure ependymoma morphology ($p < 0.001$) and confined to areas with ependymoma differentiation in mixed tumors. Clinically, pure ependymoma phenotype, chr6 loss, and TERT promoter mutations were associated with shorter progression-free survival (each $p < 0.001$). Conclusion: Our results suggest that subependymomas may acquire genetic and epigenetic changes throughout tumor evolution giving rise to subclones with ependymoma morphology (resulting in mixed tumors) that eventually overpopulate the subependymoma component (pure PFSE ependymomas).

Keywords: Subependymoma – posterior fossa – DNA methylation

P096

Incidence, clinicopathologic and genetic characteristics of mismatch repair gene mutated glioblastomas

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Background: Next Generation Sequencing (NGS) is commonly per-

formed for the integration of genetic profile in diagnosis of brain tumors. Glioblastoma (GBM) tends to recur after surgery, radiotherapy and temozolomide-based chemotherapy. Studies on implying immunotherapy in GBM treatment via mismatch repair (MMR) gene status was little and very limited. Objective: The purpose of this study was to investigate incidence, genetic and morphologic characteristics of the MMR gene mutated glioblastomas. Methods: From the file of targeted NGS data on brain tumors, a total of 282 GBMs was collected and categorized into GBMs with or without MMR gene alterations. Authors compared clinicopathological and genetic alterations between two groups. Results: MMR-gene alterations were found in 32 of 282 GBMs (11.3%), of which three cases had three or more mutations. Among MMR gene alterations, the single nucleotide variant was the most common and the most of mutations were germline but there was also amplification of MLH1 and PMS2 genes. Clinicopathological features were not significantly different between these two groups, but MGMT methylation and high tumor mutation burden (> 20) were different among two groups of GBM. Conclusion: MMR-gene alterations in GBM is not uncommon and seems to be germline mutation. Subsets of gene alterations were different among two groups of GBM. Since there were no evident morphologic changes, careful analysis of NGS study on MMR gene could be needed for future patient selection for immunotherapy.

Keywords: Glioblastoma – DNA mismatch repair gene – next-generation sequencing

P097

Distribution and characterization of *IDH1* G105G (rs11554137) SNP in a consecutive series of adult diffuse gliomas

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Background: *IDH1* G105G (rs11554137) SNP has been suggested as a potential unfavorable prognostic marker in acute myeloid leukemia, while available data concerning adult diffuse gliomas are limited. Objective: To assess the prognostic significance of the *IDH1* rs11554137 in a cohort of adult diffuse gliomas and to verify the potential associations with other molecular hallmarks. Methods: *IDH1* rs11554137 status was assessed by Sanger sequencing. Clinico-pathological and molecular data were retrieved from the relevant records at the Città della Salute e della Scienza di Torino University Hospital and the Piedmont Cancer Registry. Results: 279 adult diffuse gliomas were analyzed (median age: 62 years, range: 21 – 83) including 14 and 4 grade II and III IDHmut and 1p/19q codeleted oligodendrogliomas (O II and O III), 12 and 4 grade II and III IDHmut astrocytomas (A-IDHmut II and III), 14 and 16 grade II and III IDHwt astrocytomas (A-IDHwt II and III), 4 IDHmut glioblastomas and 211 IDHwt glioblastomas (GBM-IDHmut and GBM-IDHwt). *IDH1* rs11554137 was observed in 1 O II, 2 A-IDHmut II, 3 A-IDHmut III, 2 A-IDHwt III and 32 GBM-IDHwt. No difference in distribution of rs11554137 was observed among oligodendrogliomas, IDH-mut astrocytomas and IDH-wt diffuse gliomas ($p = 0.22$) or based upon MGMT promoter methylation status ($p = 0.46$ and $p = 0.36$ and in IDH-mut and IDH-wt gliomas). Presence of rs11554137 did not affect PFS (logrank, $p = 0.91$) or OS (logrank, $p = 0.14$). Conclusion: The *IDH1* G105G (rs11554137) SNP did not result to be associated with either *MGMT* promoter methylation status or patients' outcome.

Keywords: Diffuse gliomas – IDH1 – SNP – rs11554137 – neuro-oncology

P098

Alterations of RECQL4 and related helicases in glial neoplasms

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Introduction: RECQL4 is a ubiquitously expressed protein that belongs to the RecQ Helicase family. RECQL4 plays a role in maintaining the integrity of the genome and regulating DNA replication. Our prior work reported gene variants of RECQL4 in NF1-associated tumors, sometimes in association with abnormal telomeres. However, the relevance of gene variants in gliomas at large is unclear. Methods: Publicly available databases of genetic data were analyzed for alterations in RECQL4 and other related helicases (WRN, BLM). Immunohistochemistry using the anti-RECQL4 antibody was performed on whole tissue sections and tissue microarrays from gliomas. Immunoreactivity was recorded. The H score (% of cells positive x intensity) was used to characterize tissue microarrays. Results: Analysis of the cBIOPORTAL© database of gliomas demonstrated RECQL4 mutations in 8/794, BLM mutations in 5/794, and WRN mutations in 2/794. 35 gliomas were identified with RECQL4 gene variants by next-generation sequencing. 7 of these gene

variants were possibly pathogenic/somatic. IHC studies of whole tissue sections showed consistent nuclear staining of neurons, a small subset of subcortical glial cells, and glioblastomas. The expression was partially lost in an anaplastic astrocytoma with combined RECQL4 and NF1 mutations. 208 glial tumors of various types and grades were studied using tissue microarrays. The median H score was 120 for grades I and II, 180 for grade III, and 160 for grade IV. Conclusion: Genetic alterations in RECQL4 and related helicases occur in a subset of glial neoplasms. RECQL4 protein expression may be increased in high-grade gliomas and decreased in low-grade gliomas.

Keywords: RECQL4 – gene variants – IHC – gliomas

P099

The multi-target small-molecule inhibitor SB747651A shows in vitro and in vivo anticancer efficacy in glioblastomas

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Introduction: Most glioblastomas harbor several different genetic alterations in major cell signaling pathways, leading to tumor progression and treatment resistance. Single-targeted therapies against these alterations have thus far failed to show major clinical value. Objectives: The aim of this study was to investigate the anticancer effects of SB747651A, a multi-target small molecule inhibitor, targeting the RAS-MAPK and AKT-PI3K kinases AKT, MSK1/2, RSK1/2, and p70S6K in glioblastomas. Methods: Three patient-derived glioblastoma cultures were treated with 5 or 10 µM SB747651A. Exposed spheroids were subject to assays investigating cell viability (propidium iodide), apoptosis (Caspase 3/7), spheroid formation capacity, chemo sensitivity (temozolomide), and migration. An orthotopic xenograft model was used for investigation of in vivo efficacy. Results: Exposed cells showed increased cell death and apoptosis in a dose-dependent manner across all cell cultures ($p = 0.001$). Combination treatment with SB747651A and temozolomide resulted in significant concentration-dependent synergistic effects on cell death. The fraction of spheroid-initiating cells was significantly reduced in treated cells (1 in 12 cells) compared to controls (1 in 5 cells; $p = 0.001$), and the growth rate of treated tumor spheroids was reduced by up to 47% ($p = 0.001$). Exposed spheroids showed reduced cell migration in 2 of 3 cell lines, and survival of tumor bearing mice was significantly prolonged after SB747651A treatment (128 days vs. 112 days, $p = 0.015$). Conclusion: SB747651A treatment has shown promising in vitro results by targeting mechanisms with high relevance for the progression and treatment resistance of glioblastomas. SB747651A treatment significantly prolonged survival tumor bearing mice, and showed no adverse effects.

Keywords: Glioblastoma – small-molecule-inhibitor – MAPK – stemness – metabolism

P100

Myxopapillary ependymomas comprise two subgroups with distinct age, histomorphology, DNA methylation, gene expression, and clinical outcome

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DNA methylation profiling has emerged as a convincing tool to classify tumors of the central nervous system. However, for ependymomas of the lower spinal cord, neither histology nor DNA methylation can currently reliably predict progression-free survival (PFS). To establish a clinically relevant classification, we analyzed 185 tumors classified as myxopapillary ependymoma (MPE) based on DNA methylation. Histology, global DNA methylation, copy number alterations, and global gene expression were correlated with clinical parameters and PFS. Patients with molecularly defined MPE ranged from 5 – 81 years of age, 58% being male. 55% of the tumors were initially diagnosed as myxopapillary ependymoma (WHO grade I), 34% as ependymoma (WHO grade II), 4% as anaplastic ependymoma (WHO grade III), and 7% with other diagnoses. 97% of the tumors with available material (n = 89) immunohistochemically expressed HOXB13, a feature that was not detected in tumors assigned to the methylation groups of

spinal subependymomas (n = 4), ependymomas (n = 15), or *NMYC*-amplified ependymoma (n = 5). Based on DNA methylation, copy number alterations, and global gene expression, our series comprised two subgroups: MPE-A occurred in younger patients and were significantly enriched with tumors demonstrating papillary morphology and *MGMT* promoter methylation. These tumors occurred at lower parts of the spinal cord, could not be totally resected in many patients, and frequently relapsed. On the other hand, MPE-B included a significantly higher number of tumors with an initial diagnosis of ependymoma (WHO grade II) and tanycytic morphology. Patients within this subgroup had a significantly better outcome with a 5-year PFS of more than 90%.

Keywords: Myxopapillary ependymoma – DNA methylation – histology outcome

P101

Long-term survival and extracranial spread in a patient with a rare variant of malignant glioma

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Introduction: We present a case of a long-term malignant glioma survivor. The male patient was diagnosed with glioblastoma multiforme in 1997 and was treated with surgical resection, as well as radio- and chemotherapy. There was no sign of tumor recurrence until 2019, when the patient developed diplopia and subsequent imaging showed a tumor in his right orbita, ethmoid cells and nasal cavity. Methods: Tissue samples from 1997, as well as 2019 and 2020 have been examined histologically. Results: The tumor represents a malignant glioma which morphologically fulfills the criteria for glioblastoma WHO grade IV. However, the indolent clinical course and extracranial growth is not consistent with the diagnosis of glioblastoma. The genetic and epigenetic features support that this tumor is not a classical glioblastoma and must represent a rare variant of malignant glioma which is not classifiable with the present diagnostic tools. This case illustrates that morphology, even when typically for a glioblastoma is not always enough to obtain an accurate diagnosis in gliomas.

Keywords: Malignant glioma – glioblastoma multiforme – long-term survival – extracranial metastasis

P102/WS 8.5

Establishment of Droplet Digital™ PCR (ddPCR™) for rapid molecular diagnostics of brain tumors

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Introduction: Molecular markers provide important information related to the classification of brain tumors and the individualized prediction of a patient's response to therapy and prognosis. Therefore, it is particularly important to robustly detect diagnostically relevant genetic alterations with high sensitivity and specificity. Objectives: To facilitate the detection of brain tumor-associated genetic alterations in a time- and cost-saving manner, we aimed to establish molecular diagnostic assays based on the QX200™ Droplet Digital™ PCR (ddPCR™) system. Methods: We used ddPCR™ to detect diagnostically relevant brain tumor-associated mutations, such as hot spot mutations in the TERT promoter, IDH1 and BRAF genes, as well as copy number alterations, such as 1p/19q codeletion and amplification of selected oncogenes. This digital PCR-based technique is characterized by a high sensitivity and reproducibility to detect rare alleles and copy number variations. The principle of ddPCR™ is the generation of a large number of small partitions ("droplets"). Following end-point-PCR, fluorescence of the droplets is counted and a Poisson statistics is applied that allows for the absolute measurement of nucleic acid concentrations. Results: We established ddPCR™-based assays for the in-house detection of various brain tumor-associated genetic alterations in FFPE tumor tissues. The respective assays were validated by standard methods. Thereby, we proved ddPCR™ as a robust, rapid and reliable approach for detection of brain tumor-associated genetic biomarkers within a single day. Conclusion: Our results implicate ddPCR™ as a promising diagnostic technique that facilitates integrated histological and molecular brain tumor classification in a fast, cost-effective, and highly sensitive manner.

Keywords: ddPCR – brain tumors – molecular diagnostics

P103

BCAS1 defines a heterogenous population in 1p/19q codeleted glioma

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Introduction: Oligodendroglioma (OG) accounts for ~ 10% of dif-

fuse gliomas and is characterized by IDH1/2 mutations and 1p/19q co-deletion. They are believed to arise from glial progenitors, but studies related to new biomarkers for detection and diagnosis are scarce. In the brain, recent studies show that breast carcinoma amplified sequence 1 (BCAS1) expression defines a population of early myelinating oligodendrocytes. However, the tumorigenic potential of this cell population has never been explored in gliomas. Objectives: i) Study the distribution of BCAS1 expression on human OGs; ii) Characterize the ultrastructure of BCAS1+ cells; and iii) To evaluate whether BCAS1+ cells are proliferative. Methods: We analyzed a series of surgically removed OGs from 2015 to 2020 (n = 14). We costained BCAS1, with EGFR, Vimentin and Ki-67 by immunohistochemistry and immunofluorescence to study the distribution and proliferative status of the different cell subpopulations within OG. Furthermore, we analyzed the ultrastructure of BCAS1+ cells by immunoelectron microscopy. Results: Analyzed samples presented a subset of BCAS1+ cells constituting a heterogeneously distributed and actively proliferating population. They displayed a particular phenotype, different from that of EGFR+ cells. Further analysis of BCAS1 distribution allowed us to conclude that these cells organize either in discrete nodules or associate to EGFR+ cells in diffuse sheet-like structures. Conclusion: Our results suggest that BCAS1 is a marker defining a specific cell subpopulation within OG. As such, we propose BCAS1 as a novel biomarker to be explored in oligodendrocyte-derived gliomas.

Keywords: Oligodendroglioma – BCAS1 – glioma

P104

Immunohistochemical analysis of IDH1 and p53 in glioblastomas and its relationship to the mTOR pathway

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Background and Objectives: Classification of glioblastoma, has been based on genetic alterations, especially IDH mutation. p53 mutation is frequently seen with IDH mutant glioblastomas. The current therapy strategy for glioblastomas is, after a maximum surgical resection, conventional radiotherapy combined with temozolomide. Studies about targeting the cellular pathways altered in glioblastoma are getting more important. Methods: In our study, 349 glioblastoma cases were re-examined, who were diagnosed at Gazi University Hospital, Pathology department, between years of 2008-2017. Each case was immunohistochemically stained with IDH1, p53, RPS6, and pAKT antibodies. Results: IDH1 expression was seen in 62 (17.8%) cases; median expression of p53 was 10.0% (3.0 – 40.0). As a result of our study, IDH1 positivity was significantly associated with better outcome ($p = 0.035$). Being over 55 years old at the time of diagnosis ($p < 0.001$) and having a tumor with dense microvascular proliferation ($p = 0.023$) were associated with shorter survival. IDH1 negative tumors showed a higher expression rate for the RPS6 antibody ($p = 0.012$). The percentage and intensity of pAKT expression were not related to the IDH1 expression ($p = 0.888$). Conclusion: We observed an overexpression in RPS6 immunohistochemistry in IDH1 negative glioblastomas. This may suggest an activation of the mTOR pathway in IDH1 negative glioblastomas. Since this group of glioblastomas has a worse prognosis, it is likely that the activation of the mTOR pathway may be associated with and that there may be a favorable response to treatments for inhibition of this pathway.

Keywords: Glioblastoma – IDH1 – mTOR pathway – survival – pAKT – RPS6

P105

A small molecular assessment of tumors classified as diffuse astrocytoma at Aalborg University Hospital from 2004 to 2018

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Background: The diffuse astrocytic gliomas are classified based upon IDH-status. Several studies have documented that the IDH-wildtype grade II/III are a heterogeneous group with different clinical courses. Objectives: According to cIMPACT-NOW, update 3, from 2018 the presence of EGFR-amplification, TERT-promotor-mutation or whole chromosome 7 gain/whole chromosome 10 loss in IDH-wildtype gliomas (grade II/III), will favor the same clinical course as IDH-wildtype glioblastomas, grade IV. We wanted to examine the presence of these three criteria in a group of patients classified as diffuse astrocytoma (DA), grade II, at Aalborg University Hospital from 2004 to 2018. Methods: We collected the formalin-fixed and paraffin-embedded (FFPE) tissues from 49 patients diagnosed with DA, grade II, which were examined for the molecular markers by using Next Generation Sequencing (NGS), immunohistochemistry and fluorescence in situ hybridization (FISH). The variants of interest in NGS were TERTmut, ATRXmut and IDH1/IDH2mut, while the EGFRamp and chromosome 7/10 status were analyzed by FISH. Results: The overall proportion of IDH-wildtype tumors was 28 % (14/49). The overall proportion of patients who expressed one or more of the molecular criteria was in the IDH-wildtype tumors 78 % (11/14) and in the IDH-mutant tumors 17 % (6/35). Conclusion: We found the presence of the three described molecular criteria in a greater portion of the IDH-wildtype than the IDH-mutant. It is important to do a correlation with the clinical data to understand the full potential of reclassification of the IDH-wildtype DA.

Keywords: IDH-wildtype – TERT-mut – EGFRamp – chromosome 7/10

P106

MYB/MYBL1-altered gliomas subdivide into several groups that are morphologically and epigenetically distinct and have gene fusions of *MYBL1* or *MYB*

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Introduction: *MYB/MYBL1*-altered gliomas comprise angiocentric gliomas, isomorphic diffuse gliomas and "pediatric *MYB/MYBL1*-altered diffuse astrocytomas/gliomas". However, our knowledge on these tumors is still limited due to the small number of published cases. Moreover, studies on pediatric cases focused on molecular alterations while detailed histological and clinical data are still lacking. Also, it is unknown whether the group of *MYB/MYBL1*-altered gliomas consists of additional subgroups. Methods: We thus performed a histological assessment and molecular analyses including genome-wide DNA methylation profiling, copy number analysis and RNA sequencing on a cohort of more than 70 *MYB/MYBL1*-altered gliomas. Results: We find that, based on their DNA methylation profiles and localization, *MYB/MYBL1*-altered gliomas consist of four subgroups: supra- and infratentorial angiocentric glioma, isomorphic diffuse glioma and *MYB/MYBL1*-altered glioma NEC. While angiocentric gliomas have *MYB-QKI* fusions and rarely *MYBL1-QKI* fusions, the other two subgroups show fusions of *MYBL1* and *MYB* with various fusion partners. Histological workup indicated that there is a typical histology for each subgroup. However, especially the morphology of angiocentric gliomas may mimic histological patterns of other classes of brain tumors. Of note, many *MYB/MYBL1*-altered gliomas NEC had an increased proliferation of up to 10%. Conclusion: In summary, we show that *MYB/MYBL1*-altered gliomas separate into four molecular classes that are enriched for different types of *MYB/MYBL1*-alterations, and that show distinct morphologies. Diagnosis based on histology alone seems challenging, and additional molecular testing like DNA methylation profiling may be necessary for their identification. Workup of the clinical data and RNA-expression analyses are ongoing.

Keywords: Glioma – MYB-fusion – MYBL1-fusion – DNA methylation – RNA sequencing

P107

Prognostic role of the proliferation marker Ki-67 in glioblastomas taking tumor microenvironment, *MGMT* promoter methylation and post-surgical treatment into account

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Introduction and objectives: Survival of glioblastoma patients varies and prognostic markers are important in the clinical setting. Approaching a new era with digital pathology and improved immunohistochemical multiplexing becoming a part of daily diagnostics, our objective was to investigate the prognostic value of the Ki-67 labelling index (LI) in glioblastomas more precisely than previously by excluding proliferation in non-tumor cells from the analysis. Methods: A total of 181 glioblastoma patients (178 IDH-wildtype, 3 IDH-mutated) from a well-annotated population-based glioblastoma cohort were included. Ki-67 was identified in full tumor sections with automated digital image analysis and for the first time, the contribution from non-tumor cells was excluded using quantitative double-immunohistochemistry. For comparison of the obtained Ki-67 LI between WHO grades (II-IV), a set of 9 IDH-mutated diffuse astrocytomas and 9 IDH-mutated anaplastic astrocytomas was also stained. Results: Median Ki-67 LI was 2.7%, 6.4% and 27.5% in IDH-mutated tumors (WHO II-IV) and 24.4% in IDH-wildtype glioblastomas (WHO IV). There was no difference in median Ki-67 LI between IDH-mutated and IDH-wildtype glioblastomas ($p = 0.9$) and Ki-67 LI was not associated with survival in neither univariate (HR = 1.00, $p = 0.9$) nor multivariate analysis (HR = 1.29, $p = 0.2$). Similar results were obtained when MGMT promoter methylation status was included and IDH-mutated glioblastomas were excluded (HR = 1.26, $p = 0.2$). Conclusion: Although Ki-67 may be valuable in the differential diagnostic

setting, the prognostic value of Ki-67 LI must not be over-interpreted in the clinico-pathological context.

Keywords: Glioblastomas – Ki-67 – proliferation – prognosis – immunohistochemistry – MGMT – automated digital image analysis

P108

Beyond IDH1 R132H mutation: a Spanish cohort of non-canonical IDH mutant gliomas

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Introduction: Isocitrate-dehydrogenase (IDH) mutations arethe most important molecular alteration in infiltrating gliomas, leading to the 2016 WHO classification into "IDH-mutant" and "IDH-wt" entities. The p.R132H mutation is the most frequent change, but around 10% of IDH mutations are "non-canonical" (ncIDHmut, such as p.R132C, p.R132S, p.R132L and p.R132G in *IDH1* gene, and p.R172K, p.R172W and p.R172M in *IDH2* gene). Recent studies suggest their association with a better prognosis. The aim of this study is to describe the clinico-pathological characteristics of these non-canonical mutations in our reference population. Methods: A retrospective multicentric study was performed, involving 3 large hospitals in Spain (Hospital 12 de Octubre, Hospital de Bellvitge, Hospital Vall d'Hebron). NcIDH-mutated gliomas were identified, clinico-pathological data were collected. Results: Fifty-five gliomas with ncIDH mutations were found: 21 low-grade and 34 high-grade gliomas (21-grade III, 13-grade IV), 44 astrocytic and 11 oligodendroglial tumors. *IDH1* mutations were more frequent than *IDH2* ones (72,7% vs 27,3%), p.R132C being the most frequent change (37,5% ncIDH1mut). p.R172K represented 73.3% of ncIDH2mut cases. Interestingly this change was strongly associated with oligodendroglial lineage (81.8% R172Kmutated tumors were oligodendrogliomas). All non-canonical-oligodendrogliomas presented an *IDH2* mutation (81.8%p.R172K). No significant differences in patients' age were found between groups, with median age of 40 years old. Conclusion: Non-canonical IDH mutations represent a non-negligible event with diagnostic and prognostic implications, and their detection should be part of the diagnostic workup of infiltrating gliomas, particularly with the emergence of IDH-based clinical trials in gliomas. Interestingly in our cohort ncIDHmut in oligodendrogliomas is restricted to IDH2 (mainly p.R172K).

Keywords: Gliomas – non-canonical IDH mutations

P109

Stem cell marker ALDH1A3 is not involved in mediating radiochemoresistance while RSL-3 induces ferroptotic cell death in C6 glioma model

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Introduction: Glioblastoma multiforme remains one of the deadliest malignancies with average survival of 14,7 months and limited therapeutic options. In the popular C6 glioma model, Impact of ALDH1A3 on chemoresistance and it's fit as a stem cell marker have never been investigated. Ferroptosis, a regulated form of iron-dependent cell death, has never been investigated in the C6 glioma model either. Objectives: To assess significance of ALDH1A3 as glioma stem cell marker and in a conventional therapy setting in the C6 model, furthermore whether Ferroptosis can be induced and whether it has impact on cell viability. Methods: Construction of C6 ALDH1A3KO model by CRISPR/CAS-9 editing. Aldefluor Assay to assess ALDH1 content in C6 WT/KO. Viability assessment after Temozolomide/Irradiation treatment, Colony Forming Assay to assess tumor colony formation. Treatment with RSL-3 and Liproxstatin-1 to assess presence of Ferroptosis. Results: Aldefluor Assay identified GSC content in C6 (3,83%) and ALDH1A3 as a promising marker of GSC within the C6 tumor population. ALDH1A3KO did not increase sensitivity to TMZ or IR. Colony Formation is impaired most significantly in DEAB-inhibited C6 WT, less so in C6 ALDH1A-3KO or C6 WT (at 200 µM TMZ 12.00%, 49.48%, and 53.23% respectively). During TMZ treatment, cell death is not due to Ferroptosis, but treatment with RSL-3, a potent Ferroptosis inducer, reduces viability significantly. Conclusion: ALDH1A3 is a promising GSC marker in the C6 glioma model but its knockout does not increase chemo- or radiosensitivity. RSL-3 can induce ferroptotic cell death, its impact on conventional treatment strategies needs further investigation.

Keywords: Glioma – C6 – ALDH – ferroptosis

P110

Differential expression of genes modulating RNA-methylation in low- and high-grade glioma

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Background: Whilst substantial progress has been made in understanding the epigenetic phenomenon of DNA methylation in brain tumors, the concept of methylation of RNA has received less attention, despite being a key driver of development and cellular differentiation. N6-methylation of adenosines (m6A) is a common internal base post-transcriptional modification of messenger RNAs. Objective: This project begins the study of RNA methylation in glioma patient samples and primary glioma cell lines. We hypothesize that changes in RNA methylation are a functional mechanism for tumors to change their gene expression to evade treatment and forms part of the driving force behind heterogeneity in GBM. Methods: Expression levels of 8 key RNA methylation writer/reader/eraser genes were evaluated in LGG and GBM patient samples, and low passage primary cell lines, using rtPCR. Gene expression levels were measured in 8 GBM, 8 LGG and 3 normal brain samples, using fresh-frozen bio-banked samples. Different tumor microenvironments were sampled for each tumor. Results: There was no difference in expression between tumor regions. METTL3 and METTL14 were expressed higher in LGG than normal tissue. Expression was lower in GBM than in normal tissue across all genes. Comparison between GBM and LGG revealed that expression levels in GBM were significantly lower than in LGG for all genes except WTAP. We compare with levels of known RNA methylation modifiers on RNAseq databases. Conclusion: We demonstrate that genes regulating RNA methylation are significantly reduced in GBM versus LGG. Furthermore, YTHDF1, METTL3, and METTL14 demonstrate greater expression in LGG versus normal tissue.

Keywords: RNA methylation – glioblastoma – low grade glioma

P111/SY 6.4

Single-cell analysis of tumor-associated microglia and macrophages from human glioblastoma

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Background: Patients with glioblastoma, the most frequent and malignant primary brain tumor type, have a poor prognosis with a median survival of 14 months. A major therapeutic problem is chemoresistance. In surgically removed glioblastoma tissue, tumor-associated microglia and macrophages (TAMs) constitute up to 30 % of the cells. These cells are capable of secreting cytokines, chemokines and growth factors, thereby influencing the local microenvironment. However, the existence of different TAM subtypes and their role in glioblastoma is not fully comprehended and rarely considered therapeutically. This could explain why many clinical trials fail despite promising preclinical results. Objectives: This project aims to interrogate the existence and characteristics of different TAM subtypes in human glioblastoma biopsies in order to identify novel subpopulations and therapeutic targets. Methods: $CD11b^+$ TAMs were isolated from patient glioblastoma tissue, and single-cell RNA sequencing was performed using the 10X Genomics Chromium platform for single-cell generation and an Illumina NovaSeq6000 system for sequencing. Results: We have now sequenced 50,000 TAMs from three glioblastomas and 24,000 microglial cells from two normal brain biopsies. We were able to identify known TAM populations, but also subpopulations, which have not been described before. Analysis is ongoing. Conclusion: We have detected a TAM population which is more complex than the established M1 and M2 phenotypes, constituting a novel TAM subpopulation. We are currently investigating this finding to validate specific markers associated with this subpopulation, and for identification of novel clinically relevant targets.

Keywords: Glioblastoma – tumor-associated microglia and macrophages – TAM – single-cell RNA-sequencing

P112

The role of aldehyde dehydrogenase 1 (ALDH1) in the treatment of glioma cells with cold atmospheric plasma (CAP)

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Introduction: Despite intensive research in recent years on understanding the molecular basis of Glioblastoma multiforme (GBM), prognosis remains very poor due to treatment resistance and inevitable tumor recurrence. Several groups suggest aldehyde dehydrogenase (ALDH) isozymes as new potential

prognostic markers for cancer. Additionally, first results indicate that ALDH overexpression in GBM is associated with resistance to therapy. The effective application of cold atmospheric plasma (CAP) to various tumor cells has recently fueled the hope that CAP could be an interesting new therapeutic approach in cancer treatment. Moreover, CAP has been able to restore chemosensitivity in GBM cells. Objectives: With this work, we aim for a better understanding of the role of ALDH1 in mediating therapy resistance as well as the underlying molecular mechanisms of CAP effects in cancer treatment. Methods: C6 glioma cells were treated with CAP either alone or in combination with an ALDH inhibitor. The effects on cell viability as well as on expression and activity of ALDH1 have been investigated using standard protocols including MTT-assay, Western Blot and Aldefluor assay. Results: We observed growth inhibition in C6 cells at 90 sec and cell death at 120 sec of CAP treatment. ALDH inhibitors enhanced the effects on cell viability at longer treatment times. Although a significant decrease in ALDH activity was detected following CAP treatment, we saw no alteration of ALDH1 protein levels. Conclusion: Our results suggest CAP as a promising candidate for the therapy of GBM and demonstrate the role of ALDH1 in the induced treatment effects.

Keywords: Plasma – glioblastoma – glioma – ALDH

P113

Prognostic impact of CDKN2A/B genes deletion and MGMT promoter gene deletion on diffuse astrocytic tumors

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Introduction: In previously presented works, we demonstrated deletions of CDKN2A/B genes (delCDKN2) and deletions of MGMT promoter gene (delpMGMT) were independent negative prognostic factors in diffuse astrocytic tumors (DATs). These genetic alterations are associated with dysregulation of intracellular pathways, malignant progression and response to treatment. Objectives: This study aims to demonstrate the association between delCDKN2 and delpMGMT and the overall survival (OS) of patients with DATs. Methods: A cohort of patients from two medical institutions from Argentina with diagnostic of DATs was studied. A 2 year follow up allowed us to calculate OS. We studied delCDKN2 and delpMGMT with MLPA technique and IDH 1 and 2 gene mutations with MLPA and sequencing techniques from tumoral DNA. OS analysis was performed with Kaplan-Meier curves. Results: From 111 adult patients enrolled in the present study, 40 (36.03%) were females (mean age 53) and 71 (63.96%) males (mean age 55). According to DATs IDH status, 21 (19%) were IDHmut [8 GII, 6 GIII, 7 GIV] and 90 (81%) IDHwt [2 GII, 10 GIII, 78 GIV]. DelCDKN2 was present on 63 (56%) DATs and delpMGMT on 38 (34%) DATs. Patients with a combination of both delCDKN2 and delpMGMT had the poorest OS (mean 9.34 months), $p < 0.0034$. Conclusion: The combination of both delCDKN2 and delpMGMT proved to be a negative prognostic factor in DATs and it would contribute to the subclassification of these tumors.

Keywords: Glioma – CDKN2A/B – pMGMT – IDH – astrocytic tumor

P114

Utility of the new molecular platforms in the study of complex pediatric brain tumors

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Introduction: Currently, the available modern molecular platforms allow a more precise tumor classification and to obtain valuable prognostic and predictive data in brain tumors. These new platforms include the analysis of the DNA methylation profile and the next-generation sequencing (NGS). It is not well-known what the new pathological diagnosis technologies can contribute with respect to the more conventional molecular approach. Objectives: The objective of the work was to show the clinical utility of two different molecular platforms in the diagnosis of a series of complex pediatric brain tumor. Methods: Clinical-pathological and molecular study of a series of pediatric brain tumors undergoing s immunohistochemistry, fluorescence in-situ hybridization, DNA methylation array (Analysis using Illumina Human Methylation 850 (850k) Array and internal Classifier V11b2) or/and NGS analyses. Results: The first case was a pediatric CNS high-grade neuroepithelial tumor with BCOR alteration according to methylation classifier. The second case corresponded to a high-grade pediatric glioma with the HNRNPD-ROS1 fusion according to NGS analysis. The third case corresponded to a low-grade pilocytic astrocytoma, without BRAF-mutation or BRAF/KIAA1549-fusion, according to the methylation study, with rearrangement of the FGFR gene. Conclusion: The modern molecular platforms should be included as a useful tool in the diagnosis of complex pediatric brain tumors because it provides very valuable information.

Keywords: Pediatric brain tumors – DNA methylation-arrays – next-generation sequencing

P115/SY 6.5

Deconvolution of immunotherapy-treated glioblastoma identifies cellular heterogeneity and plasticity at the single-cell level

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Background: Glioblastoma is the most aggressive cancer originating in the brain with an average survival of 15 months. One of the characteristics of glioblastoma is the high level of intra-tumor heterogeneity, but the composition and complexity at the single-cell level is poorly understood. Objectives: Here, we aimed to assess the cellular and molecular heterogeneity of glioblastoma tumors using single-cell sequencing, and to evaluate the effects on heterogeneity in the patient after treatment with immune checkpoint inhibitors. Methods: In collaboration with the phase I trials unit at Rigshospitalet, we performed paired molecular analysis of glioma cells from primary and relapse surgery. Samples were analyzed using single-cell RNA sequencing as well as bulk RNA sequencing and whole exome sequencing. Results: We found high levels of intra-tumor heterogeneity, both with respect to the glioblastoma subtype enrichment and the cell type-specific gene expression. Using expression-based cell-type classification, we found defined recurrent cell-type populations present at both surgery time points. Moreover, changes in cellular phenotypes and proportions suggested a level of plasticity in the neoplastic cells. We found a recurrent pattern of a small population showing high levels of cell cycle activation and simultaneously expressing markers of stem cell potential. Somatic copy number analysis at the single cell level can be used to trace cellular lineages. To this end, we identified clonal and subclonal tumor cell populations in each sample. Conclusion: We found a high degree of cellular and molecular heterogeneity within and between glioblastoma patients, with phenotypic variation indicating a level of plasticity.

Keywords: Glioblastoma – single-cell RNA sequencing – immunotherapy – tumor heterogeneity

P116

Desmoplastic infantile astrocytoma/ganglioglioma: histomorphological and molecular analysis of 8 cases

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Introduction: Desmoplastic infantile astrocytoma/ganglioglioma (DIA/DIG) is a rare, low-grade tumor of infants. They have a dual morphology; areas with spindle cells in densely desmoplastic background accompanied with cellular areas composed of embryonal looking small cells. Objectives: Even though there are some recent reports, molecular features of DIA/DIGs are still not well-characterized. Here we present a series of ten DIA/DIGs of eight patients. Material and methods: Hacettepe University Hospital, Department of Pathology database was scanned for DIG/DIA. 10 tumors from 8 patients were identified. All the slides were reevaluated, and patients' demographic and clinical data were obtained from hospitals electronic patient databases. All cases were tested for BRAF V600 mutation and also screened for the presence of common fusions of tyrosine kinases (ALK, ROS1 and NTRK) by immunohistochemistry (IHC). Results: Median age at the diagnosis was 5.5 months (4 – 41 months). The male : female ratio was 1 : 3. All cases had a biphasic growth pattern with desmoplastic areas and embryonal looking areas. Two of the cases showed recurrence in less than a year and underwent re-excision. Three cases showed BRAF mutation. One BRAF mutant and one BRAF wild type case showed focal ALK expression. All cases were negative for ROS1 and only one tumor showed diffuse but weak panTRK positivity on IHC. Discussion: DIA/DIG is a low-grade tumor of early pediatric age with an indolent behavior. Although some harbor BRAF mutation, the underlying molecular alterations for most are still unknown. Our IHC screening for fusions of tyrosine kinases showed no convincing finding, so sequencing is recommended.

Keywords: DIG – DIA – infantile – BRAF

P117

Retrospective prognostic and diagnostic impact of next generation sequencing and new molecular criteria in adult glioma patients

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Introduction: The Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy (cIMPACT-NOW) has recommended to integrate molecular parameters to further refine CNS tumor classification. Objectives: In this study we aimed to reclassify a retrospective cohort of adult glioma patients according to cIMPACT-NOW recommendations (update 5 and 6). Further, we aimed to assess the impact on the overall survival (OS) and identify possible mutational subgroups. Methods: A cohort of 301 adult glio-

ma patients diagnosed at the Department of Pathology, Aarhus University Hospital between October 2017 and January 2019, was subjected to reclassification. We used a custom NGS panel consisting of 29 genes with well-known diagnostic and prognostic impact, examining these for either mutations, deletions and/or amplifications. The survival data will be described using Kaplan-Meier plots and compared using Cox proportional hazard model. Mutational subgroups will be analyzed using unsupervised hierarchical clustering. Results: 260 patients had viable tissue sufficient for NGS analysis. Reclassification resulted in a change of diagnosis in 21 cases of lower grade astrocytomas (WHO grade 2 and 3) being upgraded as glioblastoma (n = 17) or astrocytoma IDH mutant, WHO grade 4 (n = 4) and 6 cases of glioblastoma, IDH mutant being shifted to astrocytoma, IDH mutant WHO grade 4. There were no changes in oligodendrogliomas. The analysis of survival data and mutational profiles is ongoing. Conclusion: Incorporating the cIMPACT-NOW recommendations resulted in more patients being reclassified as WHO grade 4 tumors. The OS of the reclassified patients is expected to correlate with the revised diagnosis.

Keywords: cIMPACT-NOW gliomas – next generation sequencing (NGS) – reclassification – survival analysis

P118/WS 8.4

EpiDiP.org: an open access epigenomics diagnostic resource

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Introduction: Combined epigenomic and copy number profiling by microarrays have quickly become a mainstay in neuropathological tumor diagnostics since the "brain tumor methylation classifier" (DKFZ Heidelberg) was released. This supervised machine learning (ML) approach, recently extended to soft tissue tumors, is the first molecular approach that outperforms histological tumor classification. Objectives: Application of such supervised ML tools is restricted to classification of entities represented in reference datasets. We released an unsupervised ML tool for the interpretation of methylomes not yet covered by available classifiers, which facilitates tumor classification across the entire spectrum of epigenomic datasets. Methods: We chose dimension reduction by UMAP over conventional tSNE since it is less compute-intense, was ported to GPU, and delivers comparable results. Besides methylome comparison, chromosomal copy numbers are plotted with conumee. EpiDiP includes TCGA and GEO methylation datasets as well as ~ 4,600 own diagnostic cases (~ 1,800 of which were submitted anonymously), totaling > 18,500 methylomes comprising the entire spectrum of neoplasias. Results: Our free, publicly accessible R/shiny-based EpiDiP web application enables lineage interpretation, being useful for cancers of unknown primary and tumors not reliably allocated to defined methylation classes. EpiDiP also detects non-interpretable samples due to artifacts or degradation, a safety feature not yet offered by any other tool. Conclusion: EpiDiP complements the repertoire of supervised ML tools for tumor classification through a community-centered system that classifies each specimen in the context of more reference datasets than any other open-access platform.

Keywords: Epigenomic profiling – tumor classification – methylome – copy number variations – machine learning

P119

Deeper insight into intratumoral heterogeneity by MRI and PET-guided stereotactic biopsies from glioblastoma patients

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Introduction: Glioblastoma is one of the most aggressive cancers, but the molecular evolution is still not fully understood. We used PET imaging combined with deep sequencing of glioblastoma biopsies at both RNA and DNA levels to get a deeper insight into molecular evolution. In the clinical setting, PET imaging provides information about metabolically active tumor areas, but the molecular interpretation is less clear. Objective: The primary objective was to perform an intratumoral spatial comparison of biopsies from poten-

tially aggressive and less aggressive areas in glioblastomas according to PET scans. Additionally, tissue from the tumor periphery was included. Method: MRI, ^{11}C-methionine(MET) PET, and ^{18}F-FDG PET was used in combination to obtain a series of neurosurgical stereotactic biopsies from tumor areas with high MET and ^{18}F-FDG uptake (hotspot), low MET and ^{18}F-FDG uptake (coldspot), as well as tumor periphery of six glioblastoma patients. The biopsies were processed for whole genome, exome, and transcriptome sequencing. Results: Differential gene expression and gene ontology analysis showed that hotspots were enriched in gene sets associated with DNA replication, cell cycle, and notch signaling. Genome and exome analysis suggested hotspots and coldspots have similar mutational profiles. However, a limited number of hotspot-specific mutations and fusion transcripts indicated hotspot cells developed from coldspot cells that point at the potential role of hotspot driver genes in glioblastoma. Conclusion: Our findings reveal that hotspots in glioblastomas represent a more advanced stage of molecular evolution than coldspots.

Keywords: Glioblastoma – NGS – transcriptome – mutation – tumor evolution – cancer

P120

The coding and non-coding landscape of subependymal giant cell astrocytomas associated with tuberous sclerosis complex

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Background: Tuberous sclerosis complex (TSC) is a monogenetic disorder caused by inactivating mutations in *TSC1* or *TSC2*, key regulators of the mTOR pathway. In the central nervous system TSC is characterized by cortical tubers, subependymal nodules and subependymal giant cell astrocytomas (SEGAs). SEGAs may lead to impaired circulation of cerebrospinal fluid resulting in hydrocephalus and raised intracranial pressure. Purpose: The aim was to unravel the molecular mechanisms underlying SEGA growth using mRNA and small RNA sequencing. Methods: To identify novel targets, mRNA and small RNA sequencing were performed on a total of 19 SEGA samples and 8 controls. These targets were validated using Immunohistochemistry, western blots and/or quantitative PCR and further studied in primary SEGA or fetal astrocyte cell cultures. Results: We identified 9400 mRNAs and 94 microRNAs differentially expressed in SEGAs compared to control tissue. The SEGA transcriptome profile was enriched for the MAPK pathway. Analysis at the protein level confirmed that ERK is activated in SEGAs. Subsequently, the inhibition of ERK independently of mTORC1 blockade decreased efficiently the proliferation of primary patient-derived SEGA cultures. Furthermore, we found that LAMTOR1, LAMTOR2, LAMTOR3, LAMTOR4, and LAMTOR5 were over-expressed on both gene and protein level in SEGA compared to control tissue. Conclusion: Taken together, LAMTOR1-5 can form a complex, known as the "Ragulator" complex, which is known to activate both mTORC1 and MAPK/ERK pathways. Overall, this study shows that the MAPK/ERK pathway could be used as a target for treatment independent of, or in combination with mTORC1 inhibitors for TSC patients.

Keywords: SEGA – TSC – sequencing – low grade glioma

P121

Microscopic features of polymorphous low-grade neuroepithelial tumor of the young (PLNTY) in patient with epilepsy

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Introduction: The PLNTY is a new neoplasm described by J.T. Huse and coauthors in 2017. It is defined as a low-grade infiltrative tumor with presence of oligodendroglioma-like cellular pattern and intense CD34 immunoreactivity. It is regarded as a type of low-grade neuroepithelial tumor (LGNT) of children and young adults with epilepsy, described also as a kind of long-term epilepsy associated tumor (LEAT). Methods: We present a case of a 15-year-old girl with at least 5 years history of epilepsy and slow-growing lesion located in the frontal operculum of the dominant hemisphere. After gross-total resection one year follow up revealed no recurrence. Results: Pleomorphic infiltrate included, in addition to oligodendroglioma-like components, also small astrocytic areas and prevailing spindle cells element with higher cellularity, local arrangement in nests, with multiple microcalcifications. Moderately proliferating microvessels did not form glomeruli but had foci of lymphocytic infiltrations. No mitotic figures were observed and Ki-67 mitotic index did not exceed 1% even in more dense regions. There were no Rosenthal fibers or eosinophilic granular bodies. Immunohistochemical studies showed strong reactivity to GFAP, Olig2, and CD34. Antibodies against neurofilament and synaptophysin revealed presence of scattered neural/ganglionic cells. Conclusion: The neural component may be non obligatory in PLNTY or originate from infiltrated cortex. Further molecular studies are being performed to determine genetic alterations in the presented case.

Keywords: Epilepsy – pediatric brain tumor – low-grade glioma – glioneuronal tumor

P122

Remodeling of the glioblastoma immune landscape by Smac mimetic GDC-0152

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Introduction: Glioblastomas are the most aggressive brain tumors in adults, and they are characterized by an immunosuppressive microenvironment. Glioblastomas are invaded by tumor-associated macrophages (TAMs) supporting tumor growth and treatment escape. Smac mimetics are inhibitors of apoptosis antagonists, they are capable of pro-apoptotic and non-apoptotic functions in glioblastomas. They were used in combination with immunotherapy to boost the immune response but they proper immunomodulatory role has not been studied in glioblastoma. Based on our published and preliminary results, we identified Smac mimetic GDC-0152 as a potential drug candidate to promote an anti-tumoral immune response. Purpose: The aim of this study was to characterize the immunomodulatory properties of Smac mimetic GDC-0152 by using multilevel approaches. Methods: We performed clearing of the whole brain to identify leukocytes CD45+ recruitment to the tumor site, two-photon cellular dynamic imaging to analyze the dynamics of TAMs recruitment to the tumor site, and molecular mass cytometry (CYTOF) characterization to accurately phenotype the immune cells involved in GDC-0152 response. Results: Results showed that GDC-0152 increased the recruitment of CD45+ cells into the whole brain and into the tumor. GDC-0152 increased the recruitment of monocyte-derived dendritic cells (MoDCs), CD8 lymphocytes, natural killers and microglia, and decreased M2 macrophages and Border Associated Macrophages (BAMs) to the tumor site. Conclusion: Regarding these results, GDC-0152 appears to have immunomodulatory effects in favor of an anti-tumoral immunity. However, functional studies are needed to better identify the role of MoDCs, microglia and BAMs in GDC-0152 response and to point at an eventual treatment escape.

Keywords: Glioblastomas – tumor associated macrophages – Smac mimetic – microenvironment

P123/SY 3.5

Diffuse midline glioma, H3 K27M-mutant (DMG,H3) with *BRAF* V600E mutation. Presentation of two cases treated with *BRAF* V600 inhibitors

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Introduction: DMG,H3 only presented BRAF V600E mutation in eight published cases with histology of diffuse glioma, and was associated with long-term survival. Objectives: To describe the histological and imagenological features, and follow-up

of two DMG,H3 with *BRAF* V600 mutation treated with *BRAF* V600 inhibitors. Methods: Case 1: a 13-year-old boy presented an infiltrating thalamic tumor in the MRI. Case 2: a 19-year-old boy presented back pain and lower limb paresis. MRI showed infiltrating intramedullary dorsal tumors. Results: Both cases showed H3.3 K27M and *BRAF* V600E mutations. Case 1: microscopically, an astrocytic infiltrative tumor (GFAP and H3 positives, MIB1: 12%) with high cellular density and microvascular proliferation was observed. He was treated with radiotherapy plus chemotherapy. Eighteen months later he was included in a clinical trial (dabrafenib plus trametinib). Tumor size decreased four months later but after progression, the patient died 6 months later (OS: 31 months). Case 2: microscopically, an astrocytic infiltrative tumor (GFAP and H3 positives, MIB1: 20%) with mild nuclear pleomorphism and necrosis. He was treated with radiotherapy and temozolomide. After progression, he was included in Novartis compassionate use program. He died six months later (OS: 22.5 months). Conclusion: We presented two DMG,H3 with *BRAF* mutation treated with *BRAF* inhibitors, one of them showed an initial response to the therapy and survived almost 3 years. More studies are necessary to determine if BRAF inhibitors would be useful in the treatment of these tumors and improve the understanding of the significance of the presence of this mutation in DMG,H3.

Keywords: H3.3 – Braf – diffuse midline glioma

P124

Differential expression of microglia/macrophage markers, Iba1 and CD163 in glioblastoma

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Introduction: Great controversy exists surrounding the role of microglia/macrophages and their prognostic significance in malignant glioma, so great that it is reminiscent of the debate about the nature and identify of microglial cells during the last century which lasted up until 1991 when even the very existence of the microglia was questioned in a leading textbook. What we are able to recognize depends on what we examine. If the sampling is not representative of what is essential, problems occur especially when comparing markers. Methods: We have used adjacent tissue sections of IDH1-mutated and non-mutated glioblastoma cases and processed the tissue for Iba1 and CD163, markers of microglia/macrophages. The regulation of these proteins in glioblastoma is poorly understood. Thirty-seven glioblastoma cases from the Australian Genomics and Clinical Outcomes of Glioma (AGOG) tissue bank were used and digitized. Results: The striking finding of this study is that Iba1 and CD163 are not co-regulated in most glioblastoma cases. This raises important questions not only concerning the identity of the cells that are being referred to as GAMs (glioma-associated microglia/macrophages) by some authors but also the functional implications of this expression difference especially when considering the characteristic intratumoral histological heterogeneity of glioblastoma. Neuroimaging and survival correlations are currently being carried out. Conclusion: Our recently published artificial intelligence platform (doi:10.3390/cancers13040617) is expected to assist with an unbiased analysis of this complexity, allowing fine-grained expression studies of the above and additional immune markers at the cellular level using whole slide images.

Keywords: Microglia – GBM – Iba1 – CD163 – glioma

P125

Exploration of DNA methylation patterns in glioblastoma reprogrammed microglial cells

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Background: Glioblastoma (GBM) is the most aggressive primary brain tumor with a median survival rate of 14 months. The poor prognosis of GBM is highly associated with a tumor supporting microenvironment characterized by an anti-inflammatory state. Microglia, the resident immune cells of the brain, represent up to 40% of the tumor mass and

are known to be educated by GBM cells to adopt a pro-tumor phenotype. Tumor-associated microglia (TAMs) repress normal immune functions, such as phagocytosis and pro-inflammatory cytokines secretion through transcriptional reprogramming. We hypothesize that TAM reprogramming is controlled by epigenetic mechanisms, and therefore aim to understand if modifications in DNA methylation patterns can lead to pro-tumor phenotypical changes. Methods: Activated microglia are classically described as either "pro-inflammatory" M1 or "anti-inflammatory" M2 phenotypes, induced by Lipopolysaccharide (LPS) and Interleukin-4 (IL-4), respectively. In this study, we exposed murine microglial cell line BV2 to LPS, IL-4 and GL-261 GBM-conditioned medium (GBM-CM), followed by morphological characterization. Onset of activation was followed in time series every 6h to interrogate epigenetic and transcriptomic changes together with functional phagocytosis assays. We furthermore characterized the secretome present in GBM-CM and of microglia separately. Result and conclusion: After exposure to GBM-CM, microglial cells exhibited a bipolar, more elongated phenotype. We observed variations in cytokine production together with a decrease in phagocytosis compared to untreated and LPS-treated microglia. Interestingly, the secretome in GBM-CM was only slightly altered. We furthermore observed differentially methylated regions (DMRs) using the novel Infinium® Mouse Methylation BeadChip, suggesting a role of DNA methylation in microglial reprogramming.

Keywords: Microglia – DNA methylation – glioblastoma

P126

Invasion promoting genes in glioblastoma cells identified by a genome-wide CRISPR/Cas9 activation screen

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Introduction: Glioblastoma (GBM) tumor cells infiltrating the brain parenchyma escape surgical resection as well as chemotherapy and irradiation, leading to rapid tumor relapse and patient death. It is a across broad assumption that the subsets of pro-invasive tumor cells have distinct functional properties compared to cells of the tumor core. Several studies analyzed the molecular and genetic properties enabling pro-invasive GBM cells to infiltrate the surrounding brain parenchyma. Purpose: The aim of our study is to perform the first whole-genome CRISPR activation (CRISPRa) phenotypic screen aiming to identify invasion essential genes and downstream signaling pathways that facilitate GBM tumor cell invasion. Methods: The inducible *CRISPR*/*Cas9* Synergistic Activation Mediator (SAM) system is introduced in a phenotypically mild-invasive, low-passage, serum-free GBM cell line (NCH644). Specific single gene activation is mediated by a genome-wide sgRNA library targeting each human transcriptional start site with three specific sgRNAs. By defining the time point of the earliest invasive event under standardized conditions in $NCH644^{wildtype}$ cells, we may safely conclude that every pro-invasive $NCH644^{CRISPRa}$ cell appearing before this defined time point underwent a gene activation beneficial for the invasion process. Summary: Our genome-wide CRISPR/Cas9 mediated gene activation screen is not only the first CRISPRa screen aiming to identify invasion essential genes in glioblastoma but as well the first genome-wide genetic screen using the appearance of a physical property as selection criteria. This experimental approach complements existing genome-wide screens and holds the potential to identify novel candidate genes.

Keywords: Glioblastoma – invasion – gene activation – CRISPRa

P127/WS 8.6

Identification of two main subgroups among posterior pituitary tumors associated with histology, MAPK/PI3K mutations, epigenetic regulator mutations, CNV and outcome

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Introduction: Pituicytoma (PITUI), granular cell tumor (GCT) and spindle cell oncocytoma (SCO) are rare tumors of the posterior pituitary lobe. Histologically, they may be challenging to differentiate and have been proposed to represent a histological spectrum of a single entity. Methods: We performed next generation panel sequencing, copy number analysis and DNA methylation profiling on 47 tumors (14 PITUI; 12 GCT; 21 SCO) to investigate molecular features of this group of tumors and to explore possibilities of tumor subclassification. Results: Two main and one small methylation group were identified by unsupervised clustering of DNA methylation data, though the overall methylation differences were only subtle. The largest group (23 cases) contained most PITUIs and was strongly enriched for MAPK/PI3K pathway alterations (71% of sequenced cases (12/17)) and contained two cases with TERT promoter mutation. The 2nd largest methylation group (16 cases) contained most GCTs and was genetically mostly silent. The small third group was composed of four SCOs, which harbored epigenetic regulator mutations (3/4). Copy number changes were detected with varying frequencies among the groups. Outcome analysis demonstrated that the presence of any kind of chromosomal imbalances is associated with reduced progression free survival, both across all tumors and for SCOs alone. Conclusion: We demonstrate two main molecular groups among posterior pituitary tumors of which one group is enriched for potentially targetable MAPK/PI3K pathway alterations (e.g., BRAF, FGFR1). The presence of chromosomal imbalances may be associated with a higher recurrence rate and may be of particular help for estimating the clinical course of SCO.

Keywords: Pituicytoma – spindle cell oncocytoma – granular cell tumor – posterior pituitary gland neoplasms – molecular neuropathology – brain tumor

P128

Integrated analysis of molecular alterations and immune cell infiltration in primary and recurrent glioblastomas

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Introduction: Genetic alterations in glioblastomas potentially affect the immune cell composition and knowledge about their interplay may facilitate identification of patients eligible for immunotherapy. Objectives: We aimed to compare common genetic alterations between paired primary and recurrent glioblastomas and relate these findings to the immune cell composition. Methods: In a retrospective study, molecular profiling of 64 paired primary and recurrent IDH-wildtype glioblastomas was performed using next generation sequencing of 50 glioma-associated genes. DNA methylation-based subtype characterization was determined by genome-wide DNA methylation profiling. The immune cell composition was immunohistochemically evaluated using 8 main immune markers (CD4, CD8, FoxP3, CD204, PD-1, PD-L1, CTLA-4, CD86). Results: The vast majority of primary and recurrent tumors shared clonal genetic alterations in one or more of the investigated genes. Immunohistochemical analysis revealed significantly increased infiltration of $CD204^+$, $CD4^+$, $CD8^+$, and $CD86^+$cells in recurrent glioblastomas and these changes were not DNA methylation subtype specific. Integrated analysis of molecular alterations and the immune cell composition revealed a tendency for an association between two subgroups of *PTEN*-mutated and *NF1*-mutated recurrent glioblastomas and an increased accumulation of immunosuppressive $CD204^+$tumor-associated microglia/macrophages. Conclusion: Our data confirm that recurrent glioblastomas typically keep the major genetic driver mutations of the respective primary tumor but may differ with respect to their tumor microenvironment. Comparison between genetic alterations and immune cell composition showed a possible association of *NF1* and *PTEN* deficiency with the accumulation of $CD204^+$tumor-associated microglia/macrophages in recurrent glioblastomas.

Keywords: Recurrent glioblastoma – tumor infiltrating immune cells – immune checkpoint – molecular characterization

P129

Expression and prognostic value of jumonji domain-containing protein 6 (JMJD6) in a population-based cohort of IDH-wildtype glioblastoma patient

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Introduction: IDH-wildtype glioblastoma is the most frequent and aggressive type of primary brain tumor. Besides MGMT and IDH, no prognostic markers have yet been implemented in the clinico-pathological setting. Jumonji domain-containing protein 6 (JMJD6) is involved in epigenetic regulation of demethylation of histones, and its activity has been suggested to be associated with glioblastoma aggressiveness. Objectives: Our objective was to investigate the expression and prognostic potential of JMJD6 in IDH-wildtype glioblastomas. Methods: A total of 184 IDH-wildtype glioblastomas, WHO grade IV, from a population-based cohort were assessed with a chromogenic double staining with an antibody against JMJD6 and an exclusion-cocktail consisting of four antibodies (CD31, SMA, CD45 and Iba-1) recognizing components in the tumor microenvironment, enabling evaluation of tumor cells only. Stainings were quantified with a combined software- and scoring-based approach. For comparison, IDH-mutated WHO grade II, III and IV astrocytic gliomas were also stained. Results: JMJD6 was expressed in both tumor cells and non-tumor cells. The expression of JMJD6 increased with increasing WHO grade although not significantly. In multivariate analysis including age, performance status, MGMT status and post-surgical treatment high JMJD6 tumor fraction was associated with longer overall survival in IDH-wildtype glioblastomas ($p = 0.03$), but the effect disappeared when MGMT promoter status was included ($p = 0.34$). Conclusion: JMJD6 tumor cell expression in gliomas tended to increase with malignancy grade, suggesting that JMJD6 may be associated with tumor aggressiveness. JMJD6 has no prognostic value regarding overall survival in IDH-wildtype glioblastomas.

Keywords: Glioblastoma – Jumonji domain-containing protein 6 – expression – prognostic

P130

A simplified integrated molecular and immunohistochemistry-based algorithm for glioblastoma subtyping on paraffin embedded tissue sections

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Background: Glioblastomas can be classified according to their transcriptional profile in three molecular subtypes (Proneural, Mesenchymal, Classical) associated with different molecular alterations and prognosis. However, transcriptional analysis is not routinely feasible and assessment of simple tools for Glioblastomas sub-classification is required. Objectives: We propose a simplified integrated molecular and immunohistochemical approach to identify Glioblastomas subtypes in routine diagnostic material. Methods: 51 representative paraffin-embedded

samples were used for RNA-sequencing analysis by mean of a custom "Glioblastoma Transcriptional Subtypes(GliTS)" gene signature including restricted number(n = 90) of genes validated on TCGA dataset. Selected subgroup-specific gene classifiers (EGFR, TP53, ASCL1, OLIG2, PDGFRa, MET, YKL40, pNDRG1) allowed to generate immunohistochemical profiles that were integrated in a transcriptional status prediction algorithm. Results: Algorithm allowed to efficiently assign GliTS to all Glioblastomas (n = 197) maintaining high level of correspondence with TCGA dataset (79.5%, reaching 90% for Mesenchymal subgroup). GliTS distribution was in line with reported data, as its survival rate was worst for Mesenchymal. Notably, the algorithm allowed highlighting cases with comparable probability to be assigned to different GliTS, thus reflecting extreme heterogeneous phenotypes that mirrors the underlying genetic and biological tumor heterogeneity. Indeed, while Mesenchymal and Classical subgroups were well segregated, Proneural frequently showed a mixed Proneural/Classical phenotype, predicted as Proneural by algorithm, but with comparable probability to be assigned to Classical. These cases, characterized by concomitant high expression of EGFR and Proneural biomarkers, showed lower survival. Conclusion: A restricted panel of highly sensitive immunohistochemical markers allows to predict GliTS with high accuracy and significant association with clinical outcome.

Keywords: Glioblastoma – transcriptional sub-classification – immunohistochemistry

P131

Drug repurposing screen reveals glioblastoma cell line susceptibility to statins

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Background: The standard therapy for glioblastoma patients is tumor resection followed by radiotherapy and temozolomide chemotherapy. Although glioblastoma has been extensively molecularly profiled along with other cancers, this knowledge has not yet been translated into improved survival outcomes. We used a bioinformatics approach to identify potential novel therapeutic strategies for glioblastoma. Objectives: Comprehensive online datasets which have assessed up to 1376 cancer cell lines in multiple ways were interrogated to identify potential drug candidates for glioblastoma. Methods: Datasets included were from the cancer cell line encyclopedia (mRNA expression), the Achillies project (cell viability following Crispr-Cas9 knockout) and PRISM (drug treatment). A t-test comparing cell viability of glioblastoma cell lines versus other cancers was used to identify potential drug candidates, followed by the use of multiple statistical tools to investigate potential mechanisms of action and status of biomarkers. Results: Fluvastatin, pitavastatin and atorvastatin produced the most significant effects in glioblastoma cell lines, with both fluvastatin and pitavastatin being particularly potent. The anti-cancer properties of statins have previously been attributed to the inhibition of HMG-Coa reductase. Here, we found their effects correlated with erastin, an enhancer of ferroptosis and with gene knockout of UBIAD1, which participates in non-mitochondrial ubiquinone synthesis. These effects were both found in glioblastoma cells and other cancers with a mesenchymal-like phenotype. Conclusion: Statins appeared to be especially effective against glioblastoma lines and the effect could be linked to ferroptosis and inhibition of UBIAD1. In vitro validation of this finding is ongoing.

Keywords: Cancer – glioblastoma – bioinformatics – drug repurposing – ferroptosis

P132

The tumor microenvironment in early recurrent glioblastomas is enriched for pro-tumorigenic M2 microglia/macrophages

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Introduction: Glioblastomas have a complex tumor microenvironment, which is capable of supporting tumor growth and suppressing immune-mediated anti-tumor effects. However, not much is known about the influence of therapeutic interventions including surgery, chemotherapy and radiation on cellular phenotypes in the tumor microenvironment. In this study we focused on potential early influence of surgery on the tumor microenvironment in recurrent glioblastomas. Objectives: This study aimed to investigate phenotypic changes in tumor-associated microglia/macrophages in early vs. late recurrent glioblastomas. Methods: Tissue specimens from a set of 11 patients with matching primary and early recurrent tumors (recurrence ≤6 months after initial diagnosis) were compared to 12 patients with matching primary and late recurring tumors (recurrence 12-19 months after initial diagnosis). Double immunofluorescence stainings for the microglia/macrophage marker Iba1 were combined with different phenotypic M1 and M2 markers including CD14, CD68, CD74, CD86, CD163, CD204, and CD206. Results: The fraction of microglia/macrophages was significantly higher in reactive tumor regions of patients with early recurrence compared to matched primary tumors (30.3% vs. 21.7%, p = 0.01). Reactive tumor regions in early recurrent tumors had

significantly higher levels of M2 microglia/macrophages expressing CD204 (48.5% vs. 28.4%, p = 0.03) and CD206 (25.5% vs. 10.3%, p = 0.03) and a trending higher level of CD163 (53.8% vs. 37.6%, p = 0.09) compared to late recurrent tumors. Conclusion: Reactive tumor regions in early recurrent glioblastomas had higher fractions of microglia/macrophages with pro-tumorigenic M2 phenotypes compared to late recurrences, which suggests that the post-surgical inflammatory microenvironment supports tumor recurrence.

Keywords: Tumor microenvironment – recurrent glioblastoma – microglia/macrophages

P133

Nivolumab and bevacizumab for recurrent glioblastoma: a translational trial

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Introduction: Glioblastoma multiforme (GBM) is an aggressive brain tumor with a poor prognosis. Receiving the standard of care, the median survival is 14.6 months. We have no standard treatment for relapse and known options have limited effect. Novel treatments are necessary to improve survival and especially quality of life. Methods: We present our translational study; a phase II open label, two-armed translational study of Nivolumab and Bevacizumab for recurrent GBM, who have failed Stupp's regime. Patients are included in two arms depending on the possibility of salvage neurosurgical resection. Both arms receive Nivolumab and Bevacizumab was administered every second weekend, and the surgical arm also received Nivolumab 7 days prior surgery in order to obtain post-surgical specimens for analysis. Forty-four patients were included by January 2021; 20 in each arm (four screen-failures). In the surgical arm, 20 fresh tumor samples as well as paired tissue from the primary tumor were available. Tumor infiltrating lymphocytes (TILs) and tumor digest was produced. Results: We now investigate the lymphocyte composition and reactivity in the tumor by flow-cytometry and intracellular staining. Preliminary data on six patients identified one patient with high tumor reactivity. Analysis on 14 patients is ongoing and data will be presented. Furthermore, digital spatial profiling before and after Nivolumab treatment will be performed in order to investigate the effect of checkpoint inhibition on tumor microenvironment. Conclusion: Results from the intracellular staining will be presented at the conference.

Keywords: Glioblastoma – checkpoint inhibition – tumor reactivity – bevacizumab – tumor infiltrating lymphocytes (TILs)

P134

DNA methylation profiling for molecular classification of adult diffuse lower-grade gliomas

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Introduction: The revised WHO 2016 classification of the central nervous system (CNS) tumors incorporated molecular biomarkers for a more precise diagnosis and prognosis. Recently, DNA methylation profiling has emerged as a diagnostic tool facilitating and refining the diagnosis of many entities of CNS tumors. Objectives: We aimed to evaluate the value of using DNA methylation profiling to achieve molecular classification of diffuse lower-grade gliomas (dLGG) according to the WHO 2016 classification system. We further investigated whether methylation analysis could add improved molecular characterization of the tumors as well as to capture prognostic differences beyond the classical histological WHO grading in addition to molecular biomarkers (i.e *IDH* mutation status and 1p/19q codeletion). Methods: We retrospectively collected tumors from adult patients diagnosed with dLGG during 2007-2016 from the Västra Götaland region in Sweden and generated

genome-wide DNA methylation profiles of the tumors using the Illumina Infinium MethylationEPIC BeadChip array. Results: Of a total of 166 dLGG cases subjected to methylation profiling, 126 (76%) were assigned diagnostic methylation classes and subclasses with high prediction scores. The predicted methylation classes were strongly related to *IDH* mutations and 1p/19q codeletion status. The *IDH* wildtype tumors were further refined into methylation-based subgroups with distinct molecular signatures. Conclusion: We show that DNA methylation profiling is a reliable and robust technique for molecular stratification and prognostication of patients with dLGG, providing accurate detection of molecular biomarkers according to the WHO 2016 classification criteria.

Asgeir Store Jakola and Helena Carén share senior authorship.

Keywords: DNA methylation profiling – diffuse lower-grade glioma – DNA methylation-based classification – molecular classification – prognosis

P135/SY 6.6

Defects of mismatch repair proteins in pediatric high grade gliomas

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Background: Hetero- and homozygous germline mutations of the mismatch repair genes MLH1, PMS2, MSH2 and MSH6 cause Lynch and constitutional mismatch repair (CMMRD) syndrome, respectively. Affected CMMRD individuals are at risk to develop a variety of neoplasms including CNS tumors, particularly high grade gliomas (HGG), during childhood. Currently, few data on defects in mismatch repair proteins in children with pediatric HGG exist. Methods: A consecutive series of 79 supratentorial HGGs was screened. Tumor tissue was available in 42 patients, 5 were reclassified as non-HGGs. Immunohistochemistry with antibodies against MLH1, PMS2, MSH2 and MSH6 was performed in 37 tumors. Four patients with known CMMRD were included. The evaluation of the slides was performed blinded to the CMMRD status. Results: All four patients with known CMMRD (3 patients with PMS2, one with MSH6 mutation) were identified, showing loss of PMS2 and MSH2/MSH6, respectively. Additionally, we identified 6 patients with loss of MSH2/MSH6 staining in tumor cells, but retained staining in preexisting cells, indicating a pattern like in Lynch syndrome. NGS sequencing of these tumors revealed in 2 patients *MSH2* mutations and in one patient a hypermutator phenotype with *MSH2* and *MSH6* mutations. In 3/6 patients no mutations in the MMR genes were detectable. Conclusion: Immunohistochemical analyses of mismatch repair proteins is an effective tool to screen for patients with CMMRD and Lynch Syndrome and should be performed in HGGs to optimize treatment and offer affected families genetic counseling.

Keywords: Mismatch repair proteins – CMMRD – high grade glioma

P136

Comparison of preoperative neutrophil-to-lymphocyte ratio with survival, histological properties, and immunohistochemical parameters in glioblastomas

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Background and objectives: According to WHO consensus, glioblastoma patients' prognosis depends on the age, complete or incomplete resection, MGMT promoter methylation and IDH mutation status. Later studies had shown that preoperative high levels of neutrophile-to-lymphocyte ratio (NLR) related to the worse prognosis in some cancer types with different localizations like ovary, colorectum, breast, and pancreas. As this parameter is an indicator of the inflammatory response, it may be used to determine the prognosis of glioblastoma patients preoperatively. Methods: We have included 289 glioblastoma cases that were diagnosed between the years 2010 to 2017, in the Pathology Department of Gazi University Hospital, Ankara. Preoperative NLR levels have been calculated retrospectively. We compared the NLR ratio with the survival, the clinical properties, some certain histological parameters, the IDH1 and p53 immunohistochemical status. Results: Among 289 patients median NLR was 4.35 (SD: 7.43). Overall survival was 12.4 months. Based on the literature, NLR cut-off value determined as 3 and we compared NLR ≥ 3 group with NLR < 3. We observed no significant differences among survival data between these two groups (p: 0.119). According to the current study's results, the status of IDH1 and p53 immunohistochemical stainings was not related to patients' preoperative NLR value ($p > 0.05$). Conclusion: In our study, we observed that preoperative NLR value from peripheral blood count does not have a relation with overall survival. When compared with literature, this study has one of the larger patient groups and this may increase the reliability of statistical results.

Keywords: Glioblastoma – neutrophile-to-lymphocyte ratio – survival

P137

High mitochondrial DNA copy number is associated with better prognosis in young adult glioblastoma

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Introduction: Glioblastoma (GB) is the most common and aggressive form of glioma. GB frequently displays chromosome (chr) 7 gain, chr 10 loss and/or *EGFR* amplification. In young adults, *IDH1/2* mutations are associated with better prognosis. In children, histone *H3* gene mutations portend dismal prognosis. Novel reliable prognostic markers are needed in *IDH*- and *H3*-wildtype GB. High mitochondrial DNA copy number (mtDNA-CN) has been associated with longer survival in some cancers. The aim of this work was to assess the prognostic value of mtDNA-CN in adult GB. Methods: mtDNA-CN was assessed with real-time quantitative PCR in 232 adult GB. Results: 153/232 GB (66%) displayed chr7+/chr10-/*EGFR*amp, 23/232 GB (9.9%) harbored *IDH1/2* mutation and 3/232 GB (1.3%), *H3* mutation. 53/232 cases (22.8%) had no key genetic alterations. The mtDNA/nuclear DNA ratio ranged from 28 to 3882.4 (median 237.7). The ratios were subdivided into 2 groups: "low" (ratio < median, n = 116) and "high" (ratio > median, n = 116). There was no significant difference in overall survival between the two groups. In the "young adult" group (age < median age of 56.6 years, n = 117), the overall survival was significantly longer in the "high" vs "low" subgroup (27.3 vs 15 months, p = 0.0203). In the "older adult" group (age > median age, n = 115), the overall survival was significantly longer in the "low" vs. "high" subgroup (14.5 vs 10.2 months, p = 0.0116). Oxidative metabolism, linked to high mtDNA levels, decreases tumor aggressiveness and promotes cell differentiation. Conclusion: High mtDNA-CN was significantly associated with better overall survival in young adult GB.

Keywords: Glioblastoma – glioma – mitochondrial DNA – prognosis – metabolism

P138

Automated analysis of the heterogeneity of histological glioblastoma slides using neural networks

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Introduction: Modern machine learning-based methods have an enormous potential for the automated analysis of medical images and are successfully used in neuropathology. Glioblastoma multiforme (GBM) are very heterogeneous brain tumors that differ in morphological and genetic aspects. Therefore, subtypes with different prognoses are described (classical, mesenchymal, proneural, and occasionally neural subtypes). Objectives: This study investigates if neural networks can automatically detect and classify GBMs and measure the heterogeneity of GBMs to support prognosis estimates in a clinical setting. Methods: 58 GBM tissue samples (IDH-wild-type) were cut and stained with hematoxylin and eosin. 54 reference slides from brain autopsies served as controls. After digitalization, the tumorous lesions were labeled and separated from the surrounding brain tissue. Initially, a Convolutional Neural Network (CNN) will be trained for the delamination of GBM tissue from healthy tissue. Then, an additional CNN will be trained to identify the degree of morphological heterogeneity of glioblastoma. The training will be guided by immunohistochemical methods. Results: Ninety-two images (~ 1 TB of data) were processed to generate ~ 300.000 training samples per image. The output is a probability distribution over different classes. Firstly, the classes will be GBM and non-GBM, secondly the classes will be over the tumor heterogeneity, where the results are presented as color-coded images. Conclusion: We present an innovative method for GBM detection and classification. This method enables automated tumor detection and classification to increase the accuracy of individual prognosis estimates.

Keywords: Glioblastoma – heterogeneity – neural networks – expert systems

P139

Expression of glucose transporters GLUT 1, 3, and 4 in glioblastoma

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Introduction: Cancer cells have accelerated metabolism and often high glucose requirements. Glucose transport across glioblastoma cells plays a crucial role in maintaining their enhanced glycolysis. The up-regulation of specific glucose transporters (GLUT) represents one of the mechanisms of metabolic adaptation. GLUTs constitute also the potential therapeutic target. Purpose: Assessment of GLUT1, GLUT3, and GLUT4 protein expression in glioblastoma tissue in the context of topography and patho-clinical data. Methods: 126 cases of archival glioblastoma samples from the patients aged 27 to 84 years, including 10 IDH-mutated tumors were examined. Tumor histology was characterized according to neoplastic cell morphology and angiogenic phenotype (simple, pathological). GLUT1, GLUT3, GLUT4 expression was assessed immunohistochemically on prepared tissue microarrays. Slides were microscopically examined and analyzed with the Olympus CellSense program. The topography and intensity of immunoreactivity were assessed with their own scale. Results: Glioblastoma neoplastic cells expressed GLUT1 and GLUT3 in membranous and cytoplasmic fashion, with an intensified reaction in perinecrotic areas. GLUT1-positive cells concentrated around thrombosed blood vessels. Endothelial cells expressed GLUT1, but not in microvascular proliferations. GLUT3 was positive in endothelial cells in normal vessels, within microvascular parietal proliferations, and surrounding glioma cells. GLUT4 expression in neoplastic cells was cytoplasmic without specific topography, vessels were negative. 70% of cases were GLUT1 high, 60% GLUT3 high, and 20%- GLUT4 high. GLUT1 and 3 were correlated. Conclusion: GLUT1 and GLUT3 expression is frequent in glioblastoma tissue with zonal hypoxia- dependent topography and relation to angiogenic phenotype. GLUT4 expression is relatively low.

Keywords: GLUT 1,3,4 receptors – glioma metabolism – hypoxia

P140

The molecular study of the MMR deficient high-grade gliomas

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Background and purpose: Mismatch repair (MMR)-deficient brain tumors are rare and can be germline-deficient or sporadic. Here, we report MMR-deficient high-grade brain tumors to define clinicopathological and molecular character. Patients and methods: The materials were nine primary brain tumors with MMR gene mutations; four glioblastomas [GBMs]; one gliosarcoma, two astrocytomas, IDH-mutant, WHO grade 4; one diffuse midline glioma, H3 K27M-mutant; and one medulloblastoma with extensive nodularity. The patient's age ranged from 1 to75-year-old. The male-to-female ratio was 2 : 1. Next-generation sequencing (NGS) studies with targeted gene panels, immunohistochemistry, and microsatellite instability tests were carried out. Results: NGS revealed multiple pathogenic and a variant of unknown significance (VUS) mutations, suggesting a high tumor mutation burden. Two patients developed the MMR-deficient gliomas after concurrent chemotherapy and radiotherapy (CCRT with temozolomide) for initial GBM. Mutations in *MLH1*, *MSH2*, and *MSH6* were found in 5, 2, and 2 cases, respectively. One case was Lynch syndrome. The most common accompanying pathogenic mutations were in *NF1, TP53*, *PIK3CA*, *CDKN2A, ARID1A, APC,* and *KRAS.* These MMR-deficient brain tumors showed frequent multinucleated giant cells, although they were not present in all cases. The tumor cells also showed loss of nuclear expression of MMR proteins, and 67% (4/6) of tumors were microsatellite instability (MSI)-high, consistent with MMR-deficient tumors. Conclusion: Identification of MMR-deficient primary brain tumors is necessary because they are responsive to immunotherapy and require genetic counseling and follow-up of family members. Our findings could help improve the characterization of such tumors and the development of better treatments.

Keywords: High grade glioma – Lynch syndrome – MMR deficiency – microsatellite instability

P141

Phospholipase C β1: a new potential prognostic biomarker in glioblastoma multiforme

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Background: Glioblastoma multiforme (GBM) is the most common malignant brain tumor in adults. Unfortunately, today the main therapies are based on highly invasive procedures that are not effective and cannot cure definitively the tumor. Several studies have shown the centrality of phosphoinositides (PIs), and in particular of phospholipase C β1 (PI-PLCβ1) in the regulation of many mechanisms within the Central Nervous System such as cell adhesion, migration and cell cycle that, interestingly, are altered in GBM. In silico studies demonstrated that PLCβ1 gene expression is inversely

correlated with the gliomas' pathological grade, suggesting PLCβ1 as a potential prognostic factor and novel signature gene in the molecular classification of high-grade gliomas. Objectives: This study aims to determine the pathological impact of PLCβ1 in GBM patients' samples and in engineered GBM cell lines. Methods: PLCβ1 and its mediators' gene expression were analyzed through qPCR. Epithelial-mesenchymal transcription (EMT) markers and the main survival pathway targets were evaluated by Western Blot. Migration and Invasion were carried out through transwell and wound healing assays. Results: This study confirmed that PLCβ1 gene expression is lower in 20 GBM samples compared to healthy controls. Moreover, PLCβ1 silencing in U251MG and U87MG cells, leads to an increase in cell migration, invasion, EMT and in the activation of survival pathways. Conclusion: These data confirm that PLCβ1 is involved in GBM pathogenesis and a complete understanding of its role may be strategic from both pathological and clinical point of view.

Keywords: PLCbeta1 – Glioma – biomarker

P142

Clinico-imagistic features of glioblastomas at the pathology department of the SCJU Târgu Mureș

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Introduction: Glioblastomas are the most malignant cerebral tumors. Magnetic resonance imaging (MRI) is routinely used in diagnosis, characterization, and clinical management of glioblastomas. Usually, MRI scans of patients with glioblastomas show the central area of necrosis, surrounded by a contrast-enhancing ring with highly dense neoplastic cells and peripheral vasogenic edema. The radiological parameters are able to predict the tumor recurrence and patients survival. Objectives: The aim of this study is to correlate the clinical parameters with radiological parameters. Methods: In this retrospective study, we included 34 cases of glioblastomas that were diagnosed between 2016 and 2017 at the Pathology Department of County Emergency Clinical Hospital of Targu Mures. Clinical parameters like gender, age, and localization of the tumor as well as magnetic resonance parameters were collected. Descriptive statistics was used for data analysis. Results: More than half of the cases with glioblastoma have been diagnosed in 2016. Patients ranged in age from 8 to 79 years, with more than half older than 30 years. The Male/Female ratio was 1. Majority of studied cases are characterized by necrosis (79%), perilesional edema between 0.1 – 2 cm (76%), and a midline shift up to 10 mm (58%). Clinical data were not statistically significantly correlated with MRI parameters. Conclusion: The tumor is diagnosed more frequently in patients between 31 and 60 years and there are no gender differences. It develops more frequently in the right cerebral hemisphere and in the temporal lobe were related with greater tumoral aria and peritumoral edema.

Keywords: Glioblastoma – age – localization – gender – MRI

P143

Syntenin is highly expressed in IDH-wildtype glioblastoma but not associated with prognosis

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Introduction and objectives: In IDH-wildtype glioblastoma, the most frequent and aggressive type of primary brain tumor, prognostic markers are highly needed. We investigated the expression and prognostic value of syntenin – a novel marker suggested to be associated with tumor initiation and aggressiveness – in this group of patients. IDH-mutated glioblastomas were included for comparison of syntenin expression levels. Methods: Tissue microarrays with cores from 188 patients (181 IDH-wildtype glioblastomas and 7 IDH-mutated glioblastomas) were stained with an antibody against syntenin. Expression levels were quantified by a software-based approach. Survival analyses included age, gender, performance status, post-surgical treatment and MGMT status. Results: The syntenin expression level in IDH-wildtype glioblastoma (median syntenin area fraction 16.72) was significantly higher than for IDH-mutated glioblastoma (median syntenin area fraction 2.42; $p = 0.01$). In multivariate analyses syntenin expression was not associated with overall survival (OS) in IDH-wildtype glioblastoma (HR 0.78 $p = 0.12$). In 161 patients MGMT promoter methylation status was available; syntenin was not associated with OS in these patients (HR 0.85, $p = 0,4$). In this patient group, 129 patients were treated with curative intended radio-chemotherapy. For MGMT methylated patients, median OS was 24.9 months (syntenin low) and 20.5 months (syntenin high), respectively ($p = 0.97$). For non-methylated patients, the median

OS was 15.7 months (syntenin low) and 13,4 months (syntenin high) (p = 0.39). Thus, syntenin was not associated with OS in the two subgroups. Conclusion: The expression of syntenin was significantly higher in IDH-wildtype glioblastoma than in IDH- mutated glioblastoma. Syntenin was not associated with prognosis in IDH wildtype-glioblastoma.

Keywords: Glioblastoma – syntenin – mda-9

P144

TGF-β activates pericytes via induction of the epithelial to mesenchymal transition protein SLUG in glioblastoma

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Introduction: Epithelial-to-mesenchymal transition (EMT) gene expression has been associated with increased malignancy in primary CNS tumors, however, we have previously shown that EMT factors are almost exclusively expressed by glioma-vessel-associated pericytes. Objectives: In the present study, we aimed to identify the mechanism of EMT program activation in GA-Peris and its impact on angiogenic processes. Methods: In glioma patients, vascular density and expression of the pericytic markers PDGFR-β and αSMA were examined related to the expression of EMT transcription factor SLUG and correlated with survival of glioblastoma (GBM) patients. Functional mechanisms of SLUG regulation and effects on primary human brain vascular pericytes (HBVP) were studied in vitro by measuring proliferation, cell motility and growth characteristics. Results: The number of PDGFR-β and αSMA-positive pericytes did neither change with increased malignancy nor showed an association with the survival of GBM patients. However, SLUG-expressing pericytes displayed considerable morphological changes in GBM-associated vessels, and TGF-β induced SLUG upregulation led to enhanced proliferation, motility and altered growth patterns in HBVP. Downregulation of SLUG or addition of a TGF-β antagonizing antibody abolished these effects. Conclusion: We provide evidence that in GA-Peris elevated SLUG expression is mediated by TGF-β, a cytokine secreted by most glioma cells, indicating that the latter actively modulates neovascularization not only by modulating endothelial cells, but also by influencing pericytes. This process might be responsible for the formation of an unstructured tumor vasculature as well as for the breakdown of the BBB in GBM.

Keywords: Glioma – EMT – pericytes – TGF-beta

P144Z

Pleomorphic xanthoastrocytoma – a heterogeneous entity in which pTERT mutations prognosticate shorter survival

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Background: Pleomorphic xanthoastrocytoma (PXA) in its classic manifestation exhibits distinct morphological features and is assigned to WHO grade II or WHO grade III (anaplastic PXA). Distinction from glioblastoma variants and lower grade glial and glioneuronal tumors are common diagnostic challenges. Objectives: We employed methylation-based classification of CNS tumors in order to study PXAs for their morphological and molecular parameters with clinical relevance. Methods: 140 tumors with primary histological diagnosis PXA (histPXA) were subjected to DNA methylation array analysis. Those identified with the signature of methylation class (mcPXA) were combined with other tumors with mcPXA signature but having received a divergent morphological diagnosis, altogether constituting a set of 220 tumors. Morphological, molecular and clinical parameters with relevance to PXA were analyzed. Results: Only 58 tumors morphologically diagnosed as PXA (histPXA) were assigned to the mcPXA. However, tumors with mcPXA signature included a broader spectrum of histological diagnosis, with glioblastoma constituting the most frequent histology. The presence of the canonical pTERT mutation in mcPXA designated a group of PXAs with significantly worse prognosis. Conclusion: Diagnostic evaluation of tumors within the morphological scope of PXA can be assisted by DNA methylation array analysis. Our data suggest pTERT mutation as a robust indicator for poor prognosis in PXA.

Keywords: Pleomorphic xanthoastrocytoma – pTERT – DNA methylation array profiling

P144Z.1

Immunohistochemical correlates of PARP1 with ATRX and p53 in glioblastoma

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Introduction: PARP1 inhibition is a potential therapeutic target in glioblastoma (GBM), which is a frequent and aggressive primary brain tumor in adults. Our previous bioinformatic analysis revealed the correlation between PARP1 expression and the mutation status of TP53 and ATRX genes in this tumor. Objectives: In order to validate and cross check our previous bioinformatic findings, immunohistochemical analysis of PARP1 and key glioma markers were performed on a clinical cohort. Material and methods: Formalin-fixed, paraffin-embedded samples were obtained from 60 patients (30 males and 30 female) diagnosed with GBM between 2006 and 2014 at the University of Debrecen, Institute of Pathology. Immunohistochemistry was carried out to detect PARP1, ATRX, IDH1 and p53 protein expression. Results and conclusion: PARP1 staining was primarily localized in the nucleus of tumor cells. Ninety percent (54/60) of all cases were PARP1 positive, while 10% (6/60) were negative. PARP1 IHC expression was significantly associated with the expression of p53 ($p = 0.0281$) loss of ATRX ($p = 0.002$) but not with IDH1 expression. Our IHC analysis confirmed the association between PARP1 and mutated ATRX and p53. These observations suggest that PARP1 IHC expression along with p53 overexpression and ATRX loss can be promising predictive markers for efficient PARP1 inhibition in GBM.

Keywords: Glioblastoma – PARP1 – ATRX – p53 IDH1

P144Z.2

Molecular subtype dependent expression of *PARP1* in glioblastoma – a bioinformatic analysis of the TCGA dataset

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Introduction: Increased of PARP1 expression exists in various cancers, including glioblastoma (GBM). Although PARP1 inhibition is a promising therapeutic target, no comprehensive analysis has addressed PARP1's expression characteristics regarding molecular heterogeneity in GBM. Objectives: Our aim was to evaluate PARP1's associations with GBM lineage specific markers, and its transcriptomic subtypes. Materials and methods: PARP1's somatic mutations, copy number alterations (CNAs), and mRNA expression, and clinical data were collected from the "Glioblastoma Multiforme" TCGA dataset. An integrated bioinformatic analysis was performed to evaluate PARP1's genetic signature, and prognostic role in GBM. Results and conclusion: Our analysis demonstrated that *PARP1* CNA gain and increased mRNA expression level is a characteristic of glioblastoma, particularly of its Proneural (PN) and Classical (CL) subtypes. Additionally, higher PARP1 levels exhibited an inverse correlation with patient survival ($p < 0.005$) in the CL subgroup. *ATRX* ($p = 0.006$), and *TP53* ($p = 0.015$) mutations were associated with increased *PARP1* mRNA expression. Our results support the therapeutic role of PARP inhibitors in GBM with the caveat that molecular heterogeneity needs to be taken into account.

Keywords: Glioblastoma – PARP1 – ATRX – TP53

Tumors – others

P145

Concurrent EBV positive CNS diffuse large B-cell lymphoma and peripheral T-cell lymphoma, NOS

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Introduction: A 19-year-old woman with EBV mononucleosis presented for acute respiratory failure and sepsis. She represented three months later with headaches, blurred vision, and nausea with imaging showing leptomeningeal and multiple cranial nerve enhancement and multifocal hypermetabolic pulmonary nodules. Several months of clinical improvement were followed by exacerbation of pulmonary symptoms. A kidney biopsy showed an abnormal EBV-negative T-cell population without diagnostic immunophenotypic abnormalities, consistent with a peripheral T-cell lymphoma, NOS. Two cycles of CHOEP and several cycles of GDP were given for progression with interruption by a SARS-CoV-2 infection and a left parietal infarct. An Ommaya reservoir was placed and rituximab given. On follow-up, interval increased enhancement was noted along the genu of the corpus callosum and anterior to the right lateral ventricle with stable disease present in the anterior frontal lobes. Material and methods: A left frontal lobe biopsy was routinely fixed in 10% formalin and embedded in paraffin blocks. H&E staining, immunohistochemistry (CD3, CD20, MIB1) and EBV ISH were performed. Results: Histological sections showed a discohesive population of medium to large atypical lymphoid cells with areas of necrosis. These cells stained positive for CD20 and EBV by ISH with a MIB1 proliferation index of > 90%, consistent with diffuse large B-cell lymphoma. Unfortunately, the patient developed a significant postoperative hematoma with midline shift and passed away. Conclusion: Concurrent lymphomas are rare and represent less than 5% of all lymphomas. A state of immunosuppression associated with some T-cell lymphomas leads to a prominent EBV associated B-cell proliferation.

Keywords: DLBCL – PTCL – concurrent lymphoma – EBV

P146

Intracranial myxoid mesenchymal tumor with FET/CREB fusion – a novel entity causing diagnostic challenges in neuropathology

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Introduction: Intracranial myxoid mesenchymal tumor (ICMMT) with FET-CREB family fusion is a novel entity with deceptive histomorphological features and broad spectrum of clinical presentation, causing diagnostic difficulties in daily routine. These are reinforcing the importance of a comprehensive molecular workup to yield an accurate diagnosis. Objectives: We present the histopathological and molecular features of three intracranial angiomatoid fibrous histiocytoma-like (AFH-like) tumors. Methods: Case 1, a 65-year-old male with known Hodgkin's lymphoma, presented with a tumor in the fourth ventricle. Case 2, 35-year-old male, was immunocompromised due to kidney transplantation and had a parasagittal dural lesion. Case 3 presented with a cerebellopontine angle tumor in a 45-year-old male. They were mimicking ependymoma, meningioma and schwannoma on imaging, respectively. Results: Histologically,

all three cases were heterogeneous but with vague lobular arrangement, sclerotic/hyalinized areas and pseudoangiomatous spaces. The neoplastic cells most resembled myofibroblastic or myxoma cells but plump epithelioid morphology was also present. Immunohistochemistry revealed an unusual profile with strong immunoreactivity to CD68 and desmin and variable staining for EMA, synaptophysin, GFAP, PLAP and SMA. The diagnosis of ICMMT was confirmed by the presence of EWSR1-CREB family fusion by RT-PCR. Conclusion: ICMMT may be under-recognized due to its unusual clinical presentation and morphological variability. The diagnosis requires confirmation of EWSR1 gene rearrangement. This tumor family is expected to have a benign course but only limited data is available due to its rarity.

Keywords: Angiomatoid fibrous histiocytoma (AFH) – intracranial myxoid mesenchymal tumor (ICMMT) – EWSR1 – FET – CREB1

P147

Immunodeficiency-associated lymphoproliferative disorders affecting the central nervous system

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Introduction: Immunodeficiency-associated lymphoproliferative disorders (IALD) are rare and heterogeneous lymphoid neoplasms in immunocompromised hosts. IALD are a challenge for the neuropathologist due to not suspecting that it has an effect on the central nervous system (CNS). Objectives: Due to increase of immunosuppression treatments, we want to describe the frequency of CNS pathology IALD related in our hospital by adding new cases to the literature. Methods: Retrospective review of histological diagnosis of IALD in CNS from 1993 to 2021. Description of age, gender and type of immunodeficiency. Initial clinical-radiological findings, pathological diagnosis and their outcome. Results: 12 cases were found with a mean age of 53 years at the time of diagnosis and male predominance; all were caused by secondary immunosuppression, 6 HIV related, one post-transplant and 5 were iatrogenic. Initial clinical diagnosis was brain tumor, infection or inflammation. Histologically the most common IALD was diffuse large B cell lymphoma (DLBCL) and positivity for EBV was more than 80%. One patient had radiological improvement, two remains in complete remission five years after diagnosis and eight patients died. There is no outcome information available from one patient. Conclusion: 1. IALD are a differential diagnosis to consider in immunocompromised patients with a radiological image of brain tumor. 2. Our study shows the importance of neurological follow-up in patients under immunosuppression treatment. 3. In HIV positive patients the IALD most frequent was primary CNS lymphoma as it is described in the literature. 4. High prevalence of EVB infection is associated with IALD in immunocompromised patients.

Keywords: Immunosupression – lymphoproliferative disorders – central nervous system

P148

Metastatic lung adenocarcinoma with incidental finding meningioma in a frontal lobe in a young patient

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Introduction: The incidence of two distinct tumor types occupying the same anatomical localization is extremely rare among intracranial cases. We present a rare case of coexistence of meningioma with metastatic lung cancer in a young patient. Case report: A 36-year-old male patient having no history of trauma was brought to the emergency department with epilepsy seizures. In the cranial MRI, a cystic-solid lesion which was approximately 20 mm in diameter with nodular areas in the cyst wall and had peripheral severe vasogenic edema was observed in the right frontal lobe. An operation was planned for this tumor. During the operation, besides this cystic-solid lesion, a second tumor located in the dura surrounding the same frontal lobe was detected. For the first tumor, in the microscopic examination, we obtained an epithelial proliferation that contains solid and glandular structures infiltrating the glial tissue. Immunohistochemical analysis revealed the tumor was positive for CK7, TTF1, and NapsinA. This first tumor was diagnosed as "metastasis of lung adenocarcinoma". The second tumor consisted of meningothelial cells and was accompanied by psammoma bodies for focal areas. Tumor cells stained positively with EMA and progesterone receptor. The proliferation index of this tumor was less than 1% with Ki67 staining. The second tumor was diagnosed as "meningothelial meningioma – WHO grade I". Conclusion: Incidentally, a meningioma focus was found in a patient presenting with metastatic lung adenocarcinoma. These two tumors, which were not related to each other, were accepted as "coexistence of metastatic brain tumor and meningioma".

Keywords: Brain tumor – lung adenocarcinoma – meningioma – metastasis

P149

Hypothalamic hamartoma: a case report

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Introduction: Hypothalamic hamartomas (HHs) are congenital, non-progressive, tumor-like that arise from the ventral hypothalamus and tuber cinereum. There are two different primary subgroups of hypothalamic hamartoma; pedunculated tumors are associated with pituitary axis dysfunction, specifically central precocious puberty, and intrahypothalamic tumors are associated with neurologic symptoms, including epilepsy, cognitive impairment. Objective: We aimed to present a rare case of hypothalamic hamartoma. Methods: We report a case of a 5-year-old girl with gelastic epilepsy. Magnetic resonance imaging of the brain demonstrated HH (diameter: 16 mm) at the tuber cinereum that showed no enhancement effect. The material sent to our pathology laboratory is 3 cc volume, cream-colored, multi-piece surgical material. Hematoxylin-eosin-stained preparations were prepared from formalin-fixed paraffin-embedded tissue samples. Immunohistochemical analysis was performed. Results: Cytologically normal neurons and glia showed abnormal distribution in the prepared section. Numerous neuronal nodules were observed among the glial cell. Neurofilament was present in scattered neuronal processes. Synaptophysin immunohistochemistry demonstrated diffuse production within nodules and diffuse areas. IDH1, vimentin, p53 were detected negative. Ki-67 proliferation index was determined as %1. Conclusion: Hypothalamic hamartomas are rare developmental tumors that cause seizures or pituitary axis dysfunction, usually beginning in childhood. We found this case worth presenting because hypothalamic hamartomas are rare.

Keywords: Hypothalamic hamartoma – pituitary – epilepsy

P150

Molecular pathological insights in children treated for medulloblastoma and CNS-PNET in Oslo from 2005 – 2017

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Background: An unexplained regional difference in survival was observed in two previous publications on outcome for children treated for medulloblastoma and supratentorial primitive neuroectodermal tumor (CNS-PNET) in Norway. In both publications, the biopsies were classified according to the WHO 2007 classification of CNS-tumors. Objectives: Reevaluation of all embryonal brain tumors (excluding ATRT) in pediatric patients that underwent surgery and treatment at Oslo University Hospital in 2005 – 2017 to perform a retrospective molecular based risk-stratification. Methods: Specimens from all patients < 20 years with initial diagnosis of medulloblastoma or CNS-PNET were reviewed. Molecular analyses comprised NanoString gene expression, molecular inversion probe profiling, Sanger sequencing and 850K-methylation analysis. Whole chromosomal aberration (WCA)-signatures were performed in standard-risk non-WNT/non-SHH medulloblastoma for molecular risk-stratification. Results: The initially selected 53 patients comprised CNS-neuroblastomas (n = 4), pineoblastoma (n = 3), ETMR (n = 2), medulloblastomas (n = 33), cases with insufficient material for further classification (n = 5) and non-embryonal tumor entities (n = 6). Established molecular parameters and WCA-signatures in standard risk non-WNT/non-SHH medulloblastoma allowed classification of 17 MBs as molecular high-risk. These patients had a significantly worse outcome compared to the remaining 16 medulloblastomas (OS = 52.9% vs. 87.1 p = 0.036). Stratification based on clinical risk parameters failed to reach statistical significance. Combination of clinical and molecular high-risk factors was associated with the worst outcome. Conclusion: Molecular based risk-stratification of standard risk non-WNT/non-SHH medulloblastoma in addition to established molecular parameters enabled identification of medulloblastomas with dismal prognosis. Our cohort demonstrated a high number of standard-risk non-WNT/non-SHH medulloblastoma with molecular high-risk profile, which might have contributed to the unfavorable outcome data.

Keywords: Medulloblastoma – molecular pathology – outcome – Norway – risk-stratification

P151

Metaplastic lipidized meningioma in a patient harboring CHEK2 germline VUS – case report and review of the literature

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Introduction: A 55 year old woman presented complaining of persistent, frontal, oppressive headaches. She had a prior history of endometrioid carcinoma of the endometrium and luminal breast cancer. An MRI was performed to rule out brain metastasis. A 4.6 cm long, intracranial, extra axial, frontal mass was found. Complete exeresis was achieved. Pathologic examinations show a firm, homogeneous, oval shaped lesion. Microscopic studies revealed a meningothelial benign neoplasm with singular features. Objectives: To present a case of metaplastic meningioma in a patient carrying a germline variant known to be related with cancer predisposition. Methods: Meningothelial cells were seen intermixed with others with adipocyte appearance. They showed an empty, mono vacuolated cytoplasm with eccentric nuclei. Bizarre, ancient, atypia was prominent. Cells resembling histiocytes, with foamy cytoplasm, were present. Hyalinized blood vessels were observed. Scant Psamoma bodies were present. Immunophenotype resulted extensively positive for vimentin. Patchy staining for EMA was documented. Ki67 labeling was low. Review of the literature on special types of meningiomas and its molecular basis was made. Pubmed was used as search engine, publications from 2010 to 2020 were considered. Results: Metaplastic lipidized meningioma diagnosis was made. Considering the patient's history, she was tested for germline mutations using a NGS cancer predisposition panel. She was found to harbor c. 320-5T > A in CHEK2. CHEK2 alterations, through BRCAness phenotype, have been linked to cancer development. It has also been specifically associated with meningiomas. Conclusion: Meningiomas have been linked to CHEK2 mutations. However, its connection with metaplastic subtype has not been previously described.

Keywords: Meningioma – CHEK2 – germline mutations – metaplastic meningioma

P152

Anaplastic ependymoma of spine in young adult male

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Background: September 2020: 29 year old male with recent histologic diagnosis of sarcoidosis involving mediastinal lymph nodes; presents with progressive cervical myelopathy involving right upper limb. MRI studies show marked diffuse swelling of cervical cord C3/4 to T1 level. Abnormal signal involving dorsal brainstem. Methods: December 2020: C5/6 laminoplasty performed and cervical cord biopsy. Histology: High-grade tumor with high N:C ratio, hyperchromatic nuclei, lack of prominent nucleoli, variable cytoplasm. Forming sheets, trabecular with possible micro-rosettes, the latter mimicking multinucleated cells. High mitotic count. No biphasic pattern. Lack of fibrillar background, no RF or EGBs. No necrosis or obvious endovascular proliferation. Immunohistochemistry: Positive for GFAP (moderate, diffuse), EMA (cytoplasmic dot-positivity), CD99, p53. Negative for Synaptophysin, BFAF V600E, D240, Cam 5.2, IDH-1, c-myc, S-100. INI-1 expression preserved. Ki-67 index around 30%. FISH studies: MYCN not amplified. Diagnosis: Anaplastic ependymoma of spinal cord - WHO Grade III. Without MYC amplification. Literature reviewed includes "Multifocal intradural extramedullary anaplastic ependymoma of the spine" Ananya Chakravorty et al, Journal of Spine Surgery 2017; 3: 727-731. Conclusion: This is a rare entity, with an unusual radiology finding and appears unrelated to sarcoidosis. The differential diagnoses considered were metastasis, lymphoma, high-grade glioma and embryonal tumor.

Keywords: Anaplastic ependymoma – spine – c-myc

P153

Comprehensive molecular profiling of pediatric CNS tumors – experience from a single neuropathology center

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Introduction: There is increasing evidence that molecular profiling of pediatric CNS tumors not only gives a deeper insight in their underlying genomic and epigenetic mechanisms but helps improve the diagnostic practices with more reliable prognostic stratification and identification of new therapeutic targets. Objectives: The aim of this research was to investigate the best practical approach for classification of pediatric brain tumors. Methods: A retrospective study was performed on 397 pediatric cases diagnosed between 2014 and 2020. Histology of all tumors were reviewed and selected cases were investigated further by methylation array and high-throughput sequencing techniques. Results: Of 283 neuroepithelial pediatric tumors, 130 cases underwent methylation profiling and 46 cases were tested by NGS/RNA fusion panel. Methylation array successfully classified 35 embryonal tumors, 14 high-grade gliomas, 25 low-grade gliomas, 11 glioneuronal tumors and 15 ependymomas. The morphological diagnosis was confirmed in 68 cases (52%) and refined in 16 cases (12%). Molecular profiling resulted in clinically meaningful change in 13% of pathological diagnoses. NGS/RNA fusion panel detected pathogenic alterations in 63% of the samples. Histone H3-wildtype high-grade pediatric gliomas and embryonal tumors represented molecularly heterogeneous groups where molecular investigation was essential for reliable tumor classification. A subset of cases remained unsolved

despite extensive investigation and these probably represent novel entities. Conclusion: Implementation of molecular techniques in diagnostics of pediatric brain tumors is crucial to achieve a firm diagnosis, particularly in cases with unusual morphology or clinical behavior. Nevertheless, even after using these techniques the diagnosis of some cases can still remain elusive.

Keywords: Pediatric CNS tumors – methylation array – next generation sequencing – molecular profiling

P154

Intracranial myxoid mesenchymal tumor with EWSR1–rearrangement: a rare entity and source of potential diagnostic pitfalls

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Background: Intracranial myxoid mesenchymal tumor (IMMT) is a rare (< 20 cases reported) recently recognized primary central nervous system (CNS) tumor, usually diagnosed in children/young adults. Morphology and immunophenotype resembles soft tissue angiomatoid fibrous histiocytoma, myxoid variant, also with EWSR1 rearrangement. Objectives/Methods: To describe a case of IMMT and potential diagnostic pitfalls. Results: A 11-year-old female presented with vision loss and headaches. Imaging studies disclosed a superficial left frontal, well circumscribed, cystic/solid 7 cm lesion surrounded by edema and associated with midline shift. En bloc gross total resection was performed. A diagnosis of meningioma was done in an outside institute. Histologic exam revealed a capsulated spindle cell neoplasia with variable cellular density, areas of myxoid stroma and ectatic blood vessels, occasionally hemangiopericytic-like. The cells were monomorphic, slightly atypical, arranged in vaguely fascicular and microcystic patterns, with areas of collagen deposition. Mitosis, microvascular proliferation and necrosis were absent. Immunohistochemical study revealed multifocal expression of desmin and EMA, with absence of GFAP, S100, Progesterone receptor, AE1/AE3 or STAT6 expression. INI-1 expression was preserved. Ki67 labelling was very low (< 5%). Molecular studies (FISH) revealed a EWSR1 rearrangement. FUS and NR4A3 (rearranged in low-grade fibromyxoid sarcoma and extraskeletal myxoid chondrosarcoma, respectively) were not altered. Whole body CT-scan was unremarkable. A diagnosis of IMMT was performed. Conclusion: Our case supports that IMMT has distinctive morphology, immunophenotype and genetics. Given the management and prognostic implications, we emphasize the potential misdiagnosis with meningioma and the need to exclude metastasis from more frequent soft tissue myxoid tumors.

Keywords: Intracranial myxoid mesenchymal tumor – EWSR1-rearrangement – meningioma – angiomatoid fibrous histiocytoma – myxoid

P155

Electron microscopic observation of melanoma metastasis crossing the blood brain barrier

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Introduction: Brain metastasis is associated with significant morbidity and mortality. One critical stage leading to brain metastasis is the migration of cancer cells through the Blood-Brain-Barrier (BBB). Objectives: This project aims to decipher which processes metastasizing cells undergo when passing the BBB. These processes may comprise a) a paracellular migration between endothelial cells, b) a transcellular engulfment of tumor cell by endothelial processes followed by a release of malignant cells in the brain parenchyma, and c) hypothetically also a vascular occlusion by tumor cells in the capillary system with subsequent intravascular tumor growth and disruption of capillaries. Methods: To investigate this question, we established an in vitro blood-brain-barrier composed by three actors of immortalized endothelial, pericytes and astrocytic cells to study the transmigration of melanoma cells, one of the most frequent tumor cell type giving rise to brain metastases. In parallel, we are going to recreate a metastasis dissemination and extravasation event in vivo by injecting cancer cells in the heart left ventricle of mice. First, we will use correlative light and electron microscopy to decipher the metastasizing cells and characterize their mode of migration through the BBB. Conclusion: A better understanding of the distinct ways and mechanisms tumor cells take to enter the brain is necessary for the development of future prevention strategies.

Keywords: Blood brain barrier –metastasis melanoma

P156

Expression of early B-cell factor-1 in solitary fibrous tumor/hemangiopericytoma

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Introduction: Solitary fibrous tumor (SFT) and Hemangiopericytoma (HPC) are currently considered the same entity based on the common NAB2-STAT6 fusion gene. Early-B-cell-factor-1 (EBF-1) is a highly conserved transcription factor with key role in cell fate commitment of Mesenchymal Stem Cell (MSC) progenitors. We recently showed EBF-1 expression in pericytes with a possible role in pericytic phenotype cell commitment. Aim: Investigate EBF-1 expression in HPC/SFT, its possible role as distinctive diagnostic marker for HPC and SFT and correlate EBF-1 expression with histopathological features and grading. Material and methods: EBF-1, CD90 and PDGFRb were investigated by immunohistochemistry and data were validated by RT-qPCR. Results: EBF-1 was usually negative or faintly positive in SFT while constantly positive in HPC. In HPC EBF-1 expression strongly correlated with highest grade, higher cellularity and pleomorphism. Conversely, in SFT EBF-1 was constantly negative or barely positive. Data were confirmed at transcriptional level. CD90, marker of mesenchymal differentiation, was mainly expressed in SFT, while PDGFRb, marker of pericytic differentiation, was mainly expressed in HPC. Conclusion: Data suggest that EBF-1 is a sensitive diagnostic marker for HPC/SFT. Different expressions of EBF-1 in SFT and HPC suggest that, albeit considered as unique histological entities, these lesions could arise from different progenitors committed to mesenchymal (SFT) or pericytic phenotype(HPC). CD90 and PDGFRb expression corroborates this observation. Higher expression of EBF-1 in grade 2/3 HPC suggests a more immature phenotype. We hypothesize that EBF1 in MSC progenitors plays a role in triggering pericyte commitment during oncogenesis and sustained EBF1 expression impairs their final differentiation contributing to malignancy.

Keywords: Solitary fibrous tumor and hemangiopericytoma – diagnosis – grading

P157

Elucidation of the transcriptomic consequences of KLF4 and TRAF7 mutations in secretory meningiomas

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Background: Secretory meningioma (SM) is a benign histological meningioma subtype frequently located in the central skull-base, complicating surgical removal, highlighting the need of gentle targeting treatment alternatives. SM tumors arise from the arachnoid layer in the meninges and are associated with a p.K409Q mutation in KLF4 combined with different mutations in TRAF7. Objective: Identify how KLF4/TRAF7 mutations alter signaling in SM tumors. Methods: The study includes 16 FFPE samples from SM tumors and three arachnoid cysts as normal-tissue reference. All tissues were histologically confirmed, followed by genomic sequencing of KLF4, TRAF7, NF2, and TERT-promoter (C220T/C228T) using a customized NGS panel. Gene expression profiles were analyzed using NanoString Pan-cancer Progression and Pan-cancer pathways panels. Results: All 16 SM tumors presented the KLF4p.K409Q mutation, of which 12 had mutation in TRAF7 (four being p.N520S), while no cysts had mutations in these genes. Neither tumors nor cysts had NF2 or TERT-promoter mutations. PCA analysis showed differential expression profiles between the tumors and the cysts. Further analyses are planned to reveal differences in gene expression signatures of SM tumors with KLF4p.K409Q mutation and TRAF7-mutation, as compared to TRAF7-wildtype SM tumors and compared to arachnoid cysts. In vitro validation will be used as follow up. Conclusion: The KLF4p.K409Q mutation correlates more strongly with SM histology, than the TRAF7 mutations. SM tumors have gene expressions profiles that are distinct from normal arachnoid tissue. Unique data have been generated which potentially can reveal how SM specific mutations influence tumor signaling. An update will be presented.

Keywords: Meningioma – secretory – KLF4 – TRAF7

P158

Analysis of chromosomal abnormalities and of cell cycle genes alterations in a series of 48 chordomas

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Background: Chordomas are rare slow-growing sarcomas with a high

rate of recurrence. We have previously established a histopathologic grading system that correlates with progression-free survival and overall survival, including elevation of mitotic and Ki67 indices, reflecting cell cycle deregulation (Tauzie'de-Espariat A et al. 2016 PMID: 31841164). Objectives: To analyze chromosomal copy number variations and somatic single nucleotide variants of genes involved in cell cycle regulation. Methods: Forty-three chordae's including 28 clival and 15 sacral chordomas were selected from patients treated by surgery alone. Data from array comparative genomic hybridization studies and next generation sequencing of 84 genes involved in cell cycle regulation were correlated to clinicopathological data and tumor grade. Results: Clival chordomas showed significantly more gains in chromosomal regions such as 1q41-44 and 5p11, 12 while sacral chordomas showed more losses in 6p12, 6p23-25 and 19q13 regions ($p = 0.0001$). Twenty-five CNVs were significantly associated with a mitotic index ≥ 2/HPF ($p > 0.01$) and 5 with a Ki-67 index $\geq 6\%$ ($p < 0.05$). NGS analysis displayed 2 gene variants that were significantly associated with a high mitotic index and 3 variants with a high Ki67 index. Multivariate analysis of DNA variants across the combined cohort demonstrated two distinct groups of SNP associated with tumor location, such as ZBTB17 and CDR1 in the sacrum and PLK4 in the clivus, while two gene variants (MKI67 and TGFB1) were found in both locations. Conclusion: Our study shows that clival and sacral chordomas are characterized by distinct CNV and variants in genes involved in cell cycle regulation.

Keywords: Chordoma – CGH – cell cycle genes – clivus – sacrum

P159

A rare case of growth hormone secreting pituitary adenoma with ganglioglioma

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Introduction: Ganglioglioma is a generally benign lesion. The most common site for ganglioglioma is temporal lobe. Unusual locations such as adenohypophysis and neurohypophysis have also been reported. The most common tumor in this location is pituitary adenoma. Pituitary adenoma and different tumor subtype are rarely intermixed. Purpose: We present a case with a growth hormone-secreting pituitary adenoma admixed with ganglioglioma. Methods: The case is a 37-year-old female patient whose no surgical history. She presented with symptoms of acromegaly progressing for two years. MRI scanning identified a sellar mass with heterogeneous enhancement. The laboratory evaluation revealed there were elevated serum growth hormone level and insülin-like growth factor 1 level (12 µg/L and 359 ng/mL, respectively). Transsphenoidal microsurgery was performed. Results: On microscopic examination, there were two different tumors, both of which were intermixed. The first and the relatively more common tumor had neuronal and glial cells. There were dysplastic neurons and eosinophilic granular bodies in the fibrillar background. The other tumor comprised eosinophilic cytoplasm with oval uniform nuclei with occasional small nucleoli. This component showed positive staining for growth hormone, negative staining for other hormone stains. Therefore, considering the morphological and immunohistochemistry results of these neoplasms, the patient was diagnosed with growth hormone-secreting pituitary adenoma admixed with ganglioglioma. Conclusion: Ganglioglioma is only rarely located in the sella. It should be kept in mind that it can be association with pituitary adenoma or as isolated lesion.

Keywords: Ganglioglioma – pituitary adenoma – sellar region

P160

Granular cell tumor of the neurohypophysis: report of two cases

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Introduction: Granular cell tumors (GCTs) are rare soft tissue tumors. GCT can arise along the anatomical part of the neurohypophysis. Some GCTs which have nuclear pleomorphism, prominent nucleoli, multinucleated cells, and increased mitotic activity have been referred to as atypical granular cell tumors by some neuropathologists, although the clinical behavior is unclear. Purpose: We present a case diagnosed as atypical GCT of the neurohypophysis after recurrence, and we present a case diagnosed as GCT of the neurohypophysis. Methods: The first case is a 41-year-old male patient whose history included surgery for suprasellar mass 4 years earlier. He had been diagnosed with GCT. According to dynamic contrast enhanced MRI, a giant suprasellar mass measuring 45×40×36 mm was present. The second case is a 42-year-old male patient with no medical or surgical history. MRI identified sellar mass measuring 21×15 mm. Pituitary hormones levels were in normal limit in both patients. Transsphenoidal microsurgery was performed. Results: On microscopic examination, all the tumors were arranged in sheet-like pattern. The tumor cells had large, granular eosinophilic cytoplasm. In the first tumor, the granular cells showed nuclear atypia, pleomorphism and increased mitotic activity. In the second case, there was no atypical feature. The immunoprofile of these tumors showed diffuse staining for S100, CD68, PAS and negative staining for hormone stains. Therefore, considering the morphological and immunohistochemistry results of these neoplasms, the first patient was diagnosed with atypical GCT and the second patient was diagnosed with GCT. Conclusion: We described two rare cases of GCT in an uncommon site. Although the diagnosis of GCT

is difficult due to its rarity, careful morphological evaluation, as well as proper immunohistochemical studies, make this task possible.

Keywords: Granular cell tumor – atypical feature – seller region

P161

Methylation signature of atypical meningiomas

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Background: Meningiomas are one of the most common intracranial tumors in humans, however, despite benign histology, many recur. Accordingly, novel biomarkers are needed to identify cases with a more aggressive clinical course, and in that regard, methylation profiling seems promising. Objectives: The aim of this pilot study was to apply genome-wide DNA methylation analyses on a series of atypical meningiomas WHO grade 2 to find association between prognostic methylation subclasses and risk of recurrence. Methods: Twenty atypical meningiomas (formalin-fixed and paraffin embedded tissue) with early and no recurrence, respectively, underwent standardized genome-wide DNA-methylation analyses, and their signatures were correlated with the library in Heidelberg, Germany. Results: The methylation profiling revealed most cases as meningiomas, whereas subclassification according to methylation subgroups were obtained in only a few cases. Conclusion: Methylation profiling of human meningiomas stands out as a useful diagnostic tool, whereas its use as a prognostic marker needs further adjustments.

Keywords: Meningiomas – diagnosis – prognosis – methylation

P162

Epidemiology and outcomes of patients with primary central nervous system tumors managed at the King Hussein Cancer Center, 2006 – 2019

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Background: The burden of primary central nervous system (CNS) tumors in Jordan is higher than worldwide estimates. However, national data regarding epidemiology and outcomes are scarce. Objective: To study the epidemiology and outcomes of primary CNS tumors in patients managed at a comprehensive cancer center in Jordan. Methods: We performed a retrospective chart review of all Jordanian patients with a primary CNS tumor who were managed at the center between July 2006 and December 2019. We included all entities described in the 2016 CNS WHO in addition to pituitary adenoma and dermoid cyst. We used the Kaplan–Meier method to estimate the 1-year and 5-year overall survival (OS) rates for each entity. Results: We included 2094 cases. According to the ICD-O-3, 450 tumors (21.5%) were benign, 320 (15.3%) were borderline malignant, and 1324 (63.2%) were malignant. The most common site was the supratentorium (n = 1,241 [59.3%]), followed by the infratentorium, meninges, and cranial nerves (n = 540 [25.8%], 272 [13.0%], and 41 [2.0%], respectively). The most common histology was glioblastoma (n = 483 [23.1%]), followed by medulloblastoma and pilocytic astrocytoma (n = 199 [9.5%] and 166 [7.9%], respectively). Of the most common tumors, diffuse midline glioma and glioblastoma portended the lowest 1- and 5-year OS rates, respectively (38% [30 – 49%] and 11% [8 – 14%], respectively). Conclusion: We present a detailed analysis of the majority of primary CNS tumors diagnosed in Jordan between 2006 and 2019. The epidemiology and outcomes of these tumors are similar to worldwide trends.

Keywords: Central nervous system neoplasms – epidemiology – Jordan – survival analysis

P163

DNA methylation analysis reveals epigenetic regulation of neural differentiation in AT/RTs

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Introduction: DNA methylation has been identified as a key factor in oncogenesis and now analyzing methylation patterns provides new, vital information for brain tumor characterization and diagnostics. Methods: In this project, we analyzed medulloblastoma, choroid plexus, and atypical teratoid/rhabdoid tumors (AT/RTs) with public data from 450K-methylation arrays (N = 584) and gene-expression arrays (N = 110). In addition, an in-house cohort of 10 tumors was analyzed using reduced representation bisulfite sequencing (RRBS) and RNA-sequencing of matched samples. Results: We got 2325-5739 and 17175-25187 differentially methylated regions (DMRs) between tumor types in 450K array and RRBS data, respectively. AT/RTs harbored generally higher DNA methylation levels. DMRs were integrated with expression data, resulting in 44 cancer-specific genes with differential expression and DNA methylation in associated gene promoter, enhancer or genomic neighborhood. In our transcription factor (TF) binding site enrichment analysis, several TFs known to promote neural development, such as NEUROG2 and NEUROD1, were enriched in regions hypermethylated in AT/RT. Consistently, TFs, such as SMAD2, involved in the inhibition of neural development were associated with regions hypermethylated in medulloblastoma. Conclusion: This suggests that DNA methylation is regulating especially the target sites for neural regulators in AT/RT tumors, thus inhibiting neural development. Low number of genes with cancer-specific expression and methylation change is at least partly explained by the different gene expression patterns in medulloblastomas and choroid plexus tumors. Taken together, these results suggest that DNA methylation has a role as an epigenetic regulator for the oncogenesis of AT/RTs.

Keywords: Illumina – epigenetics – next-generation sequencing – NGS – pediatric

P164

Multiple site biopsies is necessary for diagnosis of primary CNS lymphoma

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Introduction: Primary central nervous system lymphoma (PCNSL) is heterogeneous in clinical and neuroimaging manifestations. Biopsy pathology is crucial for diagnosis. Objectives: Learn from typical cases to increase the biopsy positive rate in the diagnosis of PCNSL. Methods: We reviewed the neuroimaging and histology relationship in four proven PCNSL patients, 3 biopsy and 1 autopsy cases. Results: A 53-year-old female presented with progressive right hemiplegia for 9 months. MRI revealed left frontal and temporal lobe lesions. Biopsy of lesions with high metabolism in PET/CT was negative. Secondary biopsy proved diagnosis of large B cell lymphoma (LBCL). A 65-year-old male presented with right hemiplegia and dysphagia for 2 months. Biopsy of the left hemisphere lesion proved diagnosis of LBCL. A 63-year-old female presented with bilateral weakness and cognitive decline for one year. MRI revealed lesions in the bilateral frontal lobe, corpus callosum and cerebellum. Biopsy of right frontal lobe proved intravascular LBCL. An 85-year-old female presented with left hemiplegia for 10 days and bilateral diffuse lesions in frontal lobe were found. On autopsy, lymphoma tissues were widespread mixed with secondary inflammation. Some inflammatory areas were misdiagnosed for tumor on imaging when clinical analysis. Conclusion: Choose lesions with enhancement and high-metabolism in neuroimaging is not enough for accurate diagnosis. Multisite involving different levels of the lesion could increase positive rate of biopsy.

Keywords: Lymphoma – biopsy – multisite

P165

Molecular profile in intermediate-grade melanocytic neoplasm, comparative serial with uveal melanomas

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Introduction: Intermediate-grade melanocytic neoplasms (IGMN) are rare tumors of the Central nervous system. Histopathological criteria for this diagnosis and predicting their biological behavior may be challenging. Pathogenetically they are not well characterized. Similar to uveal melanoma (UM), they frequently harbor activating GNAQ or GNA11 mutations. BAP-1 loss are robust markers of poor prognosis in UM. Objectives: To assess the diagnostic and prognostic value of integrated molecular analysis in a comparative serial

composed for one IGMN, relepse, 6 UM (2 clinically aggressive) and 4 cutaneous melanoma metastases in CNS. Methods: DNA extraction was performed by the Biorobot EZ1. Polymerase chain reaction amplification of *GNAQ/GNA11* exons 4 and 5 and the use of BAP1 by immunohistochemistry was assessed in each case. Results: IGMN and the relapse samples showed BAP-1 not mutated (presence of nuclear staining). BAP-1 mutated was observed in 50% of aggressive UM and in 50% of non-aggressive UM (50%). BAP-1 not mutated was observed in all cases of cutaneous metastatic melanoma. GNAQ and GNA11 mutation should occur in all cases of MU, in IGMN and in the relapse case according to the scanty series currently published. Conclusion: BAP-1 profiles distinguished cutaneous melanoma metastases from melanocytic tumors. The absence of this mutation suggests a better clinical behavior discarding a meningeal melanoma in our patient. GNAQ/GNA11 mutation is present in a subset of primary leptomeningeal melanocytic neoplasms and these tumors genetically resemble MU. All this information also, can help in further therapeutic targets.

Keywords: Intermediate-grade melanocytic neoplasms (IGMN) GNAQ – GNA11 – BAP-1

P166

Macroscopic stimulated Raman spectroscopy: histologic verification of a classifier discriminating meningioma and dura mater

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Introduction: The quick, yet precise assessment of intraoperative samples is crucial for the residue-free tumor removal and is most frequently realized with instantaneous sections. This procedure is time- consuming and labor-intensive. Methods: An acceleration of this process could be favorable for the surgery duration and the patient's outcome. We evaluated macroscopic stimulated Raman spectroscopy (SRS) as a rapid, label-free tool for the differentiation of native meningioma and healthy dura mater tissue. We applied a fully robotized SRS system equipped with a 785 nm laser and took a macroscopic visible light image (VLI) mapping the measuring points on the examined sample. The t-distributed stochastic nearest neighbor embedding (t-SNE) algorithm was used for simple dimension-reduced cluster visualization. We trained a support vector machine (SVM) classifier using 5-fold cross- validation and applied it on newly incoming samples. Based on the classifier assessment, the VLI were color-mapped with the meningioma posterior probability (PP) at the SVM. These were afterwards compared with the corresponding hematoxylin & eosin (H&E) stained sections. Results: The t-SNE visualization forms distinct meningioma and dura mater clusters and the trained SVM classifier demonstrates high sensitivity (98.5%) and specificity (94.8%) for meningioma detection. The meningioma PP color mapping on the VLI was congruent with the histological zones on the H&E sections. Conclusion: SRS-based SVM tissue classification trained with macroscopic objects could be verified on microscopic level. Therefore, the examined SRS system could be a promising, supplementary tool for an accelerated preliminary evaluation of intraoperatively gained samples. The possibly resulting residue-free tumor resection would ameliorate a patient's outcomes.

Keywords: Stimulated Raman spectroscopy – intraoperative – meningioma – dura mater – machine learning

P167

Raman spectroscopy-based tumor entity differentiation of cryopreserved and formalin-fixed samples

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Introduction: Since numerous samples of tumor-data-banks and quick sections are preprocessed, we analyze the influence of common fixation methods on tumor samples with Raman spectroscopy (RS). Methods: We deduce the biochemical changes induced by fixation techniques with RS. The possibility to differentiate tumor entities with RS, despite fixation states, is checked. Native samples are measured directly after surgical excision by a robotized RS machine, equipped by a movable stage and a visible light image-camera. Fixations are accomplished with dry ice (cryopreservation, CP) or formaldehyde (formalin fixation, FF). Afterwards the samples are examined in neuropathology. Support vector machine is used as an unsupervised classifier. Results: Even if the formalin fixation induces changes on the biochemical level and cryopreservation forms to some extent, ice crystals, the basic biochemical structure is preserved. RS is able to discriminate between native and fixed samples with a specificity of 93.7% for CP and of 98.7% for FF. In comparison to the results of a SVM classification of native meningioma and dura mater samples (sensitivity 98.5%, specificity 94.8%), the precision is maintained for the classifying

in fixed states (CP sensitivity 96.7%, specificity 100%; FF sensitivity 95.4%, specificity 87.7%). Conclusion: This insight enables us to analyze retrospectively the tumor origin as well as extending our sample number by referring to tumor-data-banks. In this way machine learning can quickly be improved. Furthermore, RS would give a first perioperative impression of the tumor entity and could be a supportive tool for the work of the neuropathologist.

Keywords: Raman spectroscopy – fixation methods – machine learning

P168

Re-assessing the prognostic value of Ki-67 and p53 in recurrent and non-recurrent PitNETs

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Background: Pituitary Neuro-Endocrine Tumors (PitNETs) are adenohypophyseal, neoplastic growths associated with increased morbidity and mortality due to their extensive clinical and pathological presentations. The current 2017 WHO Classification recognizes a Ki-67/MIB-1 index of ≥ 3%, positive p53 immunostaining and a mitotic count of > 2 per 10 HPF as prognostic criteria indicative of PitNET invasiveness and recurrence, but studies have found this classification ineffective and too vague when considering the prognostic significance of Ki-67 and p53. Therefore, a universally accepted understanding of Ki-67 and p53's role in recurrence is needed to accurately diagnose and treat atypical PitNETs. Objectives: We aimed to identify whether the Ki-67/MIB-1 index and p53 staining in LTHT patients with PitNETs was consistent with the 2017 WHO classification and whether Ki-67 and p53 were effective predictors of recurrence based on this classification. Methods: Assessments of clinical and histological data collated over a 10-year span for all 119 cases of PitNETs in LTHT patients were conducted. Patient cases were divided into 2 groups according to the presence or absence of recurrence. Ki-67/MIB-1 indexes and the extent of p53 staining were compared between the groups. Results: The proposed Ki-67/MIB-1 index of ≥ 3% and extensive p53 staining was not associated with recurrent PitNETs. Contrastingly, non-recurrent PitNETs were associated with significantly higher Ki-67/MIB-1 indexes and more abundant p53 staining. Conclusion: There is a need to redefine characteristics indicating recurrence in PitNETs. These findings contradict the 2017 WHO Classification guidelines, suggesting that Ki-67 and p53 are ineffectual markers of PitNET recurrence.

Keywords: PitNETs – recurrence – atypical adenomas – Ki-67 – p53

P169

SHH pathway proteins expression in germ cell tumors including intracranial cases

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Introduction: Germ cell tumors (GCT) create a heterogenous group of neoplasms arising from germ cells on different phases of their differentiation and maturation, occurring in various localizations including intracranial site. Little is known regarding their pathogenesis, as a role of Sonic Hedgehog (SHH) signaling is the subject of few recent studies. Objectives: To assess expression of the SHH pathway in a set of GCT with diverse localization. Methods: TMA were constructed from paraffin blocks from 58 cases of pediatric GCT including 6 intracranial tumors (8 embryonal carcinoma, 18 germinoma type, 18 yolk sac tumors, 9 mixed GCT, and 5 teratomas). Immunohistochemistry with anti SHH, SMO, PTCH, SUFU, GLI1, G2, and GLI3 was performed, and expression was assessed with a semiquantitative scale. Results: The tumors presented expression of several SHH pathway proteins depending on their histological type and location. The lowest immunostaining was found for GLI3 and SMO. Intracranial cases showed higher GLI2, SUFU, and PTCH staining than other cases. Conclusion: SHH signaling is involved in the biology of GCT, being connected to the type, level of differentiation and tumor site.

Keywords: SHH pathway – germ cell tumors – intracranial GCT

P170

Histopathological and clinical features as prognostic factors of atypical meningiomas

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Introduction: Atypical meningiomas (AMs) have been associated with an unpredictable behavior. The post-surgical treatment of atypical meningiomas is controversial. Purpose: The aim is to analyze the correlation of clinicopathological prognostic parameters with atypical meningiomas and development of recurrence and progression-free survival (PFS). Methods: From 2010 to 2019, Seventy-nine cases with AM were reviewed histopathologically with their

clinical features. The clinical presentation, radiologic appearance and operative findings were abstracted from patients records. Pathology revision was performed based on WHO 2016 criteria. Analysis included factors such as patient age, gender, location, size of tumor, extent of surgical resection, therapy, and follow up. Statistical analysis was used to detect prognostic factors associated with recurrence and survival. Results: Recurrence occurred in 20 (27%) and mortality in 14 (18.7%) patients. The mean PFS and follow-up time were 38.9 and 44.8 months, respectively. In univariate analysis, clinical and pathological features such as age of ≤ 55 years, female sex, skull base tumor location, larger preoperative tumor size, increased mitotic count, small cells, hypercellularity, sheeting, necrosis, and dura and bone invasion were remarkable in patients with recurrence, but were not statistically significant. In multivariate analysis, increased mitotic activity and brain invasion either considered alone or combined were significantly associated with PFS ($p = 0.021$, $p = 0.004$, and $p = 0.032$, respectively). Clinical features did not significantly influence the PFS. Conclusion: This study found that recurrence could not be predicted by the presence of any of the clinicopathological features of AMs. We believe that molecular variables determined through routine neuropathological analysis will be needed in the future.

Keywords: Atypical meningioma – recurrence – progression-free survival – prognostic factors

P171

Atypical teratoid rhabdoid tumor of lateral ventricle in an adult

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Background: Atypical teratoid/rhabdoid tumor (AT/RT) is a relatively rare, highly malignant neoplasm of the central nervous system. Although the majority of cases are diagnosed in young children, there have been isolated case reports in adults. In adult cases, the most common locations reported are the sellar region and cerebral hemispheres. Purpose: We report an unusual case of AT/RT arising in the lateral ventricle in an adult patient with radiological and clinicopathological features. Methods: The patient diagnosed at Selcuk University Medical Faculty. The clinical presentation, radiologic appearance and operative findings were abstracted from patient record. Surgical specimens were routinely processed, sectioned and stained with H&E method. İmmunohistochemistry was performed. Results: A 20-year-old male presented with a history of three episodes of seizures. In addition, he complained of left frontal headache for 3-weeks prior to the onset of seizures. MRI revealed a 4.8×4.2×4 cm heterogeneously enhancing mass with solid and cystic components within the lateral ventricle. Subtotal resection of the tumor was performed via interhemispheric transcallosal approach. Histopathologically, the sections revealed sheeting of rhabdoid cells. There are areas of necrosis and frequent mitosis. Immunohistochemically tumor cells were positive with EMA, synaptophysin and focally positive to CD99. It also demonstrated a lack of nuclear INI-1 in tumor cells. The patient received chemotherapy. Conclusion: It should be noted that AT/RTs can be located in unusual intraventricular localization and may be seen in adults. The INI1 antibody can be useful tumor marker in such cases.

Keywords: Atypical teratoid rhabdoid tumor – lateral ventricle – adult

P172

The epidemiology of patients undergoing meningioma resection in Auckland, New Zealand, 2002 – 2011

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Background: Meningioma is the most common primary adult intracranial tumor. Whilst most meningiomas do not exhibit overtly malignant behavior; they can still cause significant morbidity and mortality through local effects in anatomically critical and/or surgically inaccessible locations. Objectives: This study aimed to describe the epidemiological characteristics of a 10-year cohort of patients undergoing meningioma resection at Auckland City Hospital, New Zealand. Of particular interest was whether there was any difference in meningioma incidence and recurrence rates between New Zealand Maori and Pacific Island patients compared with other ethnicities. Method: A retrospective analysis of 493 consecutive patients with pathologically confirmed meningioma over the period 1 January 2002 to 31 December 2011. Relevant clinical variables and tumor recurrence rates were recorded. All cases were reviewed histologically and classified according to the WHO 2016 classification of CNS tumors. Results: New Zealand Maori and Pacific Island patients had a significantly higher meningioma incidence than other ethnicities. Meningioma also occurred on average 7 years earlier than for European patients. On univariate analysis Pacific Island patients were at higher risk of tumor re-

currence (H.R. 1.79, p = 0.049). This risk was no longer apparent on multivariate analysis. Prognostic variables for recurrence on multivariate analysis were: WHO tumor grade, Simpson grade, tumor size and anatomic site of tumor. Conclusion: Maori and Pacific Island patients have a significantly higher incidence of meningioma at a younger age than other ethnicities. Key variables for recurrence across the cohort were: WHO grade, Simpson grade, tumor size and site of tumor.

Keywords: Meningioma – epidemiology – Maori

P173/SY 11.6

H3K27me3, PGR, SSTR2 expression and TERT mutational status in WHO-grade III meningiomas during malignant degeneration

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Introduction: Malignant meningioma (WHO-grade III) comprise 1 – 3% of all meningioma. The sequential immunohistochemical changes and the TERT mutational status during malignant progression in secondary malignant meningiomas has not yet been investigated. Objectives: To analyze tumor biopsies from consecutive operations in patients who developed a malignant meningioma. Methods: Consecutive patients at Rigshospitalet with a primary (n = 20) or a secondary (n = 20) WHO grade III meningioma from 2000 – 2018 had undergone a total of 119 operations. Tumor tissue was retrieved from 108 of these operations. Progesterone receptor (PGR), somatostatin receptor (SSTR2) and trimethylated histone H3 (H3K27me3) expression was quantified immunohistochemically. DNA was analyzed with Sanger sequencing for the C250T and C228T TERT mutation. Results: Seven patients (17.5%) had the TERT mutation (4 C228T, 1 C250T). The mutation was present already from the benign stages of the secondary malignant meningiomas. SSTR2 expression was largely retained, while PGR expression was lost during malignant degeneration. Preliminary results show that H327me3 analyses were feasible. Conclusion: Results from our population-based cohort corroborate previously reported incidence of TERT-mutations in malignant meningiomas. The mutations were present already in the original benign tumors before malignant transformation in secondary malignant meningiomas. In contrast to PGR, SSTR2 expression was not lost during malignant degeneration. Histone H3 trimethylation analyses may improve prognostication.

Keywords: Meningioma – malignant – TERT – H3K27me3

P174

The integrated diagnosis of medulloblastoma in a middle-aged, pregnant patient: a case report

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Introduction: Medulloblastoma is a primary cerebellar tumor with four histologically defined groups and four genetically defined groups. Histological subtypes are classic, desmoplastic/nodular, extensive nodularity and large cell/anaplastic. Genetic subtypes are WNT activated, SHH activated (either TP53 mutated or TP53 wildtype) non WNT/non SHH, group 3 and group 4. Methylation profiling has an important role in predicting outcomes in these patients. Purpose: We examine this case in a middle-aged, pregnant lady to highlight the diagnostic, prognostic and treatment values of the integrated diagnosis and methylation profile in medulloblastoma. Methods: Histological examination, immunohistochemistry, FISH, methylation profiling and NGS were performed. Results: The histological appearances of the excised tumor were a diffuse, small, round, blue cell mass with Homer Wright pseudorosettes. Immunohistochemistry was positive for Synaptophysin and GFAP. The integrated diagnosis was medulloblastoma WHO grade IV, histological subtype – classic medulloblastoma, molecular subtype – SHH-activated and TP53-wildtype medulloblastoma, molecular data – medulloblastoma, subclass SHH A (children and adult), no MYCN or MYC amplification, no TP53 mutation. Patient outcome in SHH-activated and TP53-wildtype is varied but it is considered to be a standard risk tumor. One study demonstrated a 5-year survival of 76% for patients with tumors of this genetic subtype. Additionally, adults with an SHH-activated tumor are more likely to have genetic alterations sensitive to SMO inhibitors. Our patient progressed to receive chemotherapy, radiotherapy and further surgery. Conclusion: Full molecular genetic workup, including methylation profiling, is essential in the diagnosis of medulloblastoma.

Keywords: Medulloblastoma, Methylation profiling, Integrated diagnosis

P175

Functional analysis of a novel *CTNNB1* mutation in pediatric medulloblastoma (MB)

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Introduction: Over the past 10 years novel insights into the biology and genomic complexity of MB has allowed the definition of specific variants and provided the basis for the identification of target genes involved in the initiation and progression of tumors. Purpose: We present a mutation in *CTNNB1* gene not previously reported in pediatric classic WNT-activated MB. Methods: A 9-year-old boy previously healthy with holocranial cephalea and MRI cerebellar tumor centered on the IV ventricle. After surgical resection MB samples were analyzed by histology, immunohistochemistry and FISH. CSF on day +15 suggested metastatic spread. Tumor DNA isolation and mutational analyses of the *CTNNB1* gene were performed. For functional analyses, we transiently transfected U2OS human osteosarcoma cells with two constructs of two β-catenin variants (one our patient's). Cellular protein extracts were analyzed by Western blot. Luciferase reporter assays were performed. Results: We detected a heterozygous c.109-111del (p.Ser37del) in the *CTNNB1* gene, resulting in the in frame deletion of a TCT codon and loss of Ser37 residue. ΔS37 β-catenin variant displayed in vitro a significant higher protein expression and significantly increased the TCF/LEF transcriptional activity at higher levels than wild type β-catenin. This mutation targets specifically the GSK-3β phosphorylation domain, resulting in its constitutive stabilization, nuclear accumulation and contribution to tumorigenesis by transcriptional activation of gene targets in WNT/β-catenin pathway. Conclusion: We present a *CTNNB1* mutation c.109-111del (p.Ser37del) not previously reported in classic WNT-activated MB. Functional analysis discloses gain-of-function properties for the novel ΔS37 β-catenin variant contributing to a more active WNT pathway and tumorigenesis.

Keywords: Medulloblastoma CTTNNB1 mutation

P176

Proliferative index like a prognostic factor for medulloblastoma. A useful tool in low and middle-income countries

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Background: Medulloblastoma is the most frequent malignant tumor in infants and children and corresponds to 20% central nervous system tumors. It is in that perspective that we proposed to find in the proliferation index evaluated with ki67 a useful and accessible prognosis cut-point that supports taking therapeutic decisions. Design: Based on the minimum and maximum values, obtained per patient from three determinations of the Ki-67 index observed independently by three pathologists. Curves estimated by the Kaplan-Meier method, log-rank tests, and Cox regression analyzes were carried out to analyze the association between cut-off points of the Ki-67 index and global survival. A p-value < 0.05 was considered for a significant difference or association. Results: Considering the maximum determinations of the Ki-67 index, the most relevant cut-off point occurred in 55% for the Ki-67, (n = 65, AUC = 0.676, 95% CI : 0.543 – 0.809, sensitivity = 62.5%, specificity = 75.8%). We found that the life span of patients in the group with Ki-67 index values less than or equal to 55% is significantly longer than that of patients in the group with Ki-67 values greater than 55%. Conclusion: We found a cut-off point of 55% or plus of proliferative index, with a prognostic value that defines aggressive behavior in medulloblastoma and shorter overall survival. We propose immunohistochemistry to ki-67 as an accessible tool present in most pathology departments in lower and middle-income countries.

Keywords: Medulloblastoma – prognosis – pathology

P177

Chordoma arising from benign notochordal tumor: a case report

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We report a case of chordoma arising from a benign notochordal cell tumor (BNCT), which was discovered in a 35-year-old male who presented with diplopia, diagnosed as 6th nerve palsy. MRI imaging showed an expansile clival lesion with sclerotic borders invading the cavernous sinus and encasing the carotid. Histopathology showed features of chordoma with areas of BNCT. This case highlights the wide differential diagnosis of clear cell tumor arising in the clival region and the importance of radio-pathological correlation.

Keywords: Chordoma – benign notochordal cell tumor

P178

Rare thyroid transcription factor 1-positive tumors of posterior pituitary gland: experience of a single institution in 20 years

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Introduction: Neoplasms from neurohypophysis are a rare group of tumors, which includes pituicytoma, granular cell tumor and spindle cell oncocytoma; Are non-endocrine low-grade tumors, which share the expression of thyroid transcription factor-1 (TTF-1). Due to the low incidence and little clinical-radiological correlation, these entities are a diagnostic challenge. Objectives: To analyze the frequency in our hospital adding new cases with a clinical radiologic histopathologic profile and their treatment outcome. Methods: Retrospective review of histological diagnosis of pituitary tumors in our institution since January 1999 until January 2021. Five cases of tumors arising in neurohypophysis were found. Description of age, gender, initial radiological diagnosis, treatment received and clinical follow up. Results: From an average of 700 pituitary surgeries per year, 0, 7% resulted in posterior pituitary tumors. Average age of diagnosis was 62; three females and two men. Clinical symptoms were related with mass effect. One case was an incidental finding in a clinical autopsy and four cases were endoscopic resection specimens, with initial diagnosis of hypophyseal adenoma and craniopharyngioma. Pathological types were two pituicytomas, one spindle cell oncocytoma, and two granular cell tumors. All cases expressed nuclear TTF-1. During follow-up two cases showed tumor persistence, one of them despite radiotherapy. Conclusion: 1. The low number of our series of cases is consistent with the literature. 2. Histopathological findings are essential for the diagnosis of posterior pituitary tumor, due to lack of specific clinical and radiological signs. 3. Low proliferation index is consistent with their benign behavior, nevertheless close follow up is recommended.

Keywords: Neurohypophyisis – TTF-1 – posterior pituitary

P179

Sonic hedgehog and Wnt signaling drives murine ocular lesions reminiscent of intraocular medulloepithelioma

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Background: Intraocular medulloepitheliomas (IO-MEPLs) are rare embryonal ocular neoplasms, which share histomorphological features with variants of embryonal tumors with multilayered rosettes (ETMR). Previous studies have demonstrated that sonic hedgehog (Shh) and Wnt signaling pathways are crucial for ETMR pathogenesis. Objectives: Our objective in this study was to examine human IO-MEPLs for SHH and WNT signaling and to explore the effect of simultaneous Shh and Wnt activation in mouse retinal precursor cells. Methods: Using Nanostring technology, gene expression data was obtained from FFPE tumor samples of 8 human IO-MEPLs, as well as 16 intracranial embryonal tumors comprising the entities ETMR and SHH-medulloblastoma (MB), WNT-MB and Group 4-MB. A tamoxifen inducible *Cre*^{ERT2}-*lox* system was utilized to activate Shh and Wnt signaling in *Rax*- or *Sox2*-expressing retinal precursor cells in a timepoint-specific manner. Results: IO-MEPLs and ETMRs displayed similar gene expression patterns and significant overrepresentation of both SHH and WNT target genes. Co-activation of both pathways in either *Rax*- or *Sox2*-positive retinal precursor cells at embryonic day E8.5 resulted in the occurrence of ocular lesions with histomorphological and immunohistochemical features reminiscent of IO-MEPLs. Conclusion: We demonstrate that human IO-MEPLs are characterized by overexpression of both WNT and SHH target genes and that coactivation of both pathways in murine retinal precursor cells drives ocular lesions with strong similarity to human IO-MEPLs. Our results may set the foundation for the first IO-MEPL mouse model and provide an experimental platform for therapeutic approaches in the future.

Keywords: Intraocular medulloepithelioma – ETMR – sonic hedgehog – wnt – mouse

P180

Axonal density in prostate cancer – morphological and quantitative study

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Introduction: Solid tumor innervation/axonogenesis is a new direction of investigation to define complex interactions between cancer cells and the microenvironment. Axonal density (AxD) is one of the parameters describing neural stroma and can be a potential marker for studies on cancer neurobiology. Objectives: Quantitative and qualitative analysis of AxD in prostate cancer clinical samples. Methods: 73 cancer (PCa) and 15 benign prostate (BP) FFPE samples were examined. The tissue sections were elaborated with TMA technology. Additionally, chosen whole tumor sections were topographically assessed. Immunohistochemistry included panneural marker PGP9.5, and TH for sympathetic fibers. AxD specified as a number of independent PGP9.5, and TH positive structures (small fibers) in 10 hot spots/200× was assessed in areas: PCa, proximal periphery (PPCa), and BP. Olympus CellSense software was used for analysis. Results: A heterogeneously distributed axonal network exists in PCa stroma, with relation to tumor histological grade. Quantitative Results: AxD TH: PCa (8.4; 95% CI: 6.9 – 10.1), BP (15.6; 95% CI: 10 – 40.8), PPCa (21.5; CI: 95% 17.9 – 29.6). AxD PGP9.5: PCa (31.5; 95% CI: 24.3 – 36), BP (38.1; 95% CI: 15.2 – 61.2), PPCa (42; 95% CI: 33.8 – 55). The statistical analysis showed: lower AxD (both markers) in cancer than the periphery, no difference AxDPCa vs BP, lower TH fibers density and their proportion to all fibers in cancer than in BP tissue. Conclusion: Prostate cancer tumor innervation is heterogenous showing lower AxD than in tumor periphery with less numerous sympathetic fibers. Complex crosstalk neural fibers – cancer cells are involved in PCa biology.

Keywords: Axonal density – nerve density – axonogenesis – neural density

P181

Copenhagen meningioma grading

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Introduction: The extent of meningioma resection is the most fundamental risk factor and exact knowledge of extent of resection is necessary for prognostication and for planning of adjuvant treatment. Currently used classifications are the EANO-grading and the Simpson grading. The former comprises radiological imaging with contrast enhanced MRI and differentiation between "gross total removal" and "subtotal removal", while the latter comprises a five-tiered differentiation of the surgeon's impression of the extent of resection. Objective: To develop an objective grading system based on microscopic analyses of resection margins and sensitive radiological analyses to improve management of follow-up, adjuvant therapy and prognostication of meningiomas. Methods: The extent of resection is defined via immunohistopathological analyses of resection margins have been used for research, but until now not implemented clinically. PET/MRI imaging with Ga-68 DOTATOC allows more sensitive and specific imaging than MRI following surgery of meningiomas. Based on the rationale of resection-margin analyses as golden standard and superior imaging performance of 68-Ga DOTATOC PET, we implement "Copenhagen grading" for meningiomas. Results: Copenhagen Grade is a combination of histopathology (0 or 1) and radiological imaging (0 or 1). It can be expressed as 0/0, 1/0, 0/1 or 1/1 depending on the diagnostic observations. Copenhagen Grading has been implemented since December 2020 as a clinical standard at Rigshospitalet, Copenhagen. Conclusion: Copenhagen Grading provides a comprehensive, logical and reproducible definition of the extent of resection. It is based on a combination of microscopic resection-margin analysis and the most sensitive and specific imaging modality available for meningiomas.

Keywords: Meningioma – neuro-oncology – neuropathology – neurosurgery

P182

Implementation of DNA methylation arrays in pediatric brain tumors classification

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Introduction: Pediatric brain tumors (PBT) represent the second most common pediatric cancer, with the highest mortality rate among childhood malignancies. Improvement of PBT diagnostic accuracy is fundamental to optimize treatment strategy. Objectives: We aimed to explore the impact of DNA methylation arrays implementation in PBT clinical practice. Methods: 350 PBT were analyzed by Illumina 850K EPIC methylation array. Low score and discordant cases were collegially reviewed. Results: Calibrated score was 0.8 or higher in 257 cases (73.4%) with diagnosis confirmation in 229 cases and accurate molecular subgroup definition in 71 of them, including cases of nonWNTnonSHH medulloblastomas, CNS neuroblastoma FOXR2 and MN1-rearranged HGNET; methylation profiling amended diagnosis in 6 cases, e.g. HGNET BCOR, anaplastic PXA, IDH-mutant glioma of the brain stem, was misleading in 5 cases, e.g. high grade gliomas classified as pilocytic astrocytomas, and inaccurate in 11 cases, e.g. supratentorial ependymoma classified as spinal ependymoma. Based on available data, pathological/methylation discordance could not be definitely addressed in 6 cases. Low scores (0.3-0.8) and no match results (< 0.3) were obtained respectively in 60 (17.1%) and 33 (9.4%) cases, which were enriched with low grade glial/glioneural tumors ($p < 0.0006$), tumors bearing translocations involving EWSR1 (e.g., EWSR1-CREM, EWSR1-PATZ1) or tyrosine kinase receptor genes (e.g. KANK1-NTRK2, ZCCHC8-ROS1) ($p < 0.0002$) and syndromic patients ($p = 0.06$). Conclusion: Methylation profiling can improve diagnostic accuracy in PBT classification, albeit with some limitations in categorizing low grade tumors and tumors arising in cancer predisposition syndromes. Low scores are often associated with rare/poorly characterized entities and should prompt further molecular investigations.

Keywords: Methylation profiling – clinico-pathological correlation – pediatric brain tumors – EWSR1-rearranged brain tumors – TK genes-rearranged brain tumors

P183

The Ki-67 proliferation index as marker of time to recurrence in intracranial meningioma

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Background: There are examples of incongruence between the WHO grade and clinical course in meningioma patients. This incongruence between WHO grade and recurrence has led to search for other prognostic histological markers. Objectives: To study the correlation between the Ki-67 proliferative index (PI), risk of recurrence and recurrence rates in meningioma patients. Methods: We prospectively collected the pathological diagnosis of 159 de novo consecutive meningiomas patients, which were followed with clinical visits until recurrence, death or emigration. We estimated the correlation between risk of recurrence and Ki-67 PI when adjusted to age at diagnosis, sex, WHO grade, extent of surgical resection and tumor location. We estimated the cumulative incidence of recurrence, when considering death without recurrence a competing risk. We report recurrence rates per 100 person-years. Results: 1%-point increase of Ki-67 PI yielded a hazard ratio of 1.12 (95% CI: 1.01 – 1.24) in a multivariate analysis. The cumulative incidence of recurrence was 3% for Ki-67 PI 0 – 4% vs. 19% Ki-67 > 4% meningiomas after 1-year, but 24% vs 35%, respectively, after 10-years. There was no significant difference in mean Ki-67 PI between non-recurrent and recurrent meningioma in a two-sample t-test ($p = 0.08$). The strongest relationship was detected between Ki-67 PI and time to recurrence: Ki-67 PI < 4% meningiomas recurred after median 4.8 years, compared to 0.60 – 0.75 years for patients with higher Ki-67 PI. Conclusion: Ki-67 PI was a marker for time to recurrence rather than a predictor of recurrence.

Keywords: Meningioma – brain tumor – ki-67 – recurrence

P184

Poor prognosis associated with TERT gene alterations in meningioma is independent of the WHO classification

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Background: *TERT* gene alterations (*TERT*-alt) have been linked to a poor prognosis in meningiomas. As incongruence between clinical course and WHO grade exists, reliable biomarkers have been sought. Objective: To investigate the effect of *TERT*-alt on recurrence and overall survival in meningioma patients. Methods: We applied the PRISMA-IPD Statement. We compiled data from eight studies and allocated patients to *TERT*-alt (n = 59) or *TERT* promoter wild-type (*TERT*p-wt, n = 618). We compared the two groups stratified for WHO grades as: incidence rates, survival probabilities and cumulative recurrences. We estimated the effects of WHO grade, age at diagnosis and sex as hazard ratios. Results: *TERT*-alt occurred in 4.7%, 7.9% and 15.4% of WHO-I/-II/-III meningiomas, respectively. The median recurrence-free survival was 14 months for all *TERT*-alt patients versus 101 months for all *TERT*p-wt patients. The hazard ratio for *TERT*-alt was 3.74 in reference to *TERT*p-wt. For all *TERT*-alt patients versus all *TERT*p-wt patients, the median overall survival was 58 months and 160 months, respectively. The hazard ratio for *TERT*-alt was 2.77 compared to *TERT*p-wt. *TERT*-alt affected prognosis independent of WHO grades. Particularly, the recurrence rate was 4.8 times higher in WHO-I/-II *TERT*-alt patients compared to WHO-III *TERT*p-wt patients. The mortality rate was 2.7 times higher in the WHO-I & -II *TERT*-alt patients compared to WHO-III *TERT*p-wt patients. Conclusion: *TERT*-alt is an important biomarker for significantly higher risk of recurrence and death in meningiomas. We propose that *TERT*-alt analysis should be implemented as a routine diagnostic test in meningioma and integrated into the WHO classification.

Keywords: Meningioma – TERT – prognosis – WHO classification

P185

Preclinical cerebral cryoablation: feasibility, safety and efficacy in non-tumor bearing pigs

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Background: Patients with brain metastases, the most common intracranial tumor, have an average survival varying from a few to 40 months. Thus, new treatment initiatives are direly needed. Cryoablation is a minimally invasive, well-tolerated and effective procedure commonly applied for treatment of renal and certain other malignancies. Objectives: We aimed to examine the clinical usefulness of this procedure in a step-by-step program starting with cerebral cryoablation in healthy pigs. Methods: In four terminal and four non-terminal non-tumor bearing pigs, we studied short- and long-term effects of cerebral cryoablation. Safety was assessed by CT, measuring macroscopic lesion size, and clinical observation of behavior, neurological deficits, and wellbeing. Effects were assessed by histological and immuno-histochemical analyses addressing structural and metabolic changes supported by additional MRI and PET in the non-terminal animals. Results: Using CT-guidance, cryoablation probes were successfully inserted without complications, and ice formation could be monitored real-time with CT. No animal developed neurological deficits or showed signs of discomfort. Histological analyses, MRI, and PET revealed both profound structural and biological damage within the lesion. MRI and PET revealed no long-term damage to healthy tissue outside the cryoablation zone in the ipsilateral or contralateral hemisphere. Conclusion: Cerebral cryoablation appears

to be a feasible, safe and controllable procedure that can be monitored successfully with CT during ablation. The net effect is a dead brain lesion without damage of nearby and remote healthy structures. Short-term changes due to cryoablation are local hemorrhage and edema; long-term effects are perfusion defects, immune system activation and astrogliosis.

Keywords: Treatment – cerebral cryoablation – pigs – immunohistochemistry – MRI – PET

P186

Prognostic value of the Ki-67 index in childhood medulloblastoma

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Background: The Ki-67 index is a predictor of prognosis in adult medulloblastoma independent of the molecular subgroup. However, the prognostic value of the marker in pediatric medulloblastoma has not been established in the context of the molecular classification. Objective: To investigate whether the Ki-67 index holds value as a marker of prognosis in childhood medulloblastoma independent of the molecular risk strata. Methods: We accessed the electronic medical records at a comprehensive cancer center in Jordan and included all pediatric patients with medulloblastoma diagnosed between 2003 and 2016. We classified all cases according to the molecular signatures and interpreted the Ki-67 index at a cutoff value of 30%. We used the Kaplan-Meier method and the log-rank test to estimate the 5-year overall survival (OS) and progression-free survival (PFS) rates and make between-group comparisons. Results: The final sample consisted of 85 patients. The mean age of the cohort was 7.7 years (range, 2.9 – 17.6 years). According to the molecular classification, 17 tumors (20.0%) were WNT-activated, 21 (24.7%) were SHH-activated, 20 (23.5%) were group 3, and 27 (31.8%) were group 4. The overall 5-year OS and PFS rates were 70.2% (SE, 5.5%) and 67.9% (SE, 5.8%), respectively. The Ki-67 index was high in 54 cases (63.5%) and low in 31 (36.5%). The Ki-67 index was not significantly related to OS or PFS overall or within any of the molecular subgroups. Conclusion: The Ki-67 index is not an independent marker of prognosis in childhood medulloblastoma.

Keywords: Ki-67 antigen – medulloblastoma – molecular pathology – pediatrics

P187

Confocal laser endomicroscopy: the distribution of fluorescent sodium in ex vivo samples of brain tumors

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Introduction: Confocal laser endomicroscopy (CLE) is an upcoming method of intraoperative tumor tissue examination in neurosurgery. Infiltrative growth can be detected on a cellular level which enables improving the identification of resection margins and defining follow-up treatments. Objectives: With this work, we aim to ease the work process of CLE by understanding the behavior of fluorescent sodium, the staining agent, and its signal enhancement in vital and avital tissue. Methods: During surgery, fluorescent sodium was applied intravenously into 40 patients, tumor tissue was sampled and examined by CLE ex vivo. A focus was set on different areas in the tumor and a comparison of staining enhancement in different entities made. Furthermore, we investigated various application times for optimal imaging. Results: Based on a collection of 4,000 images we were able to show that a healthy brain is not stained by fluorescent sodium. The transitioning zone only gives weak signals and individual tumor entities enhance the staining in different intensities. An optimal application time slot was set between 30 minutes and an hour. Conclusion: CLE could be used to define the tumor margin on a cellular level in the future. In order to implement this method, further in vivo investigation will be necessary.

Keywords: CLE – fluorescent sodium – infiltrative growth

P188

Intermediate grade melanocytoma in the context of meningeal melanocytosis – discussion of a difficult case

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Introduction: By definition, melanocytosis is a noninvasive, diffuse or multifocal proliferation of melanocytes in the subarachnoid space. Meningeal melanocytoma is the benign, localized form. Melanocytic neoplasms of the CNS are rare and while the correct diagnosis of localized lesions is histological, the diffuse forms may require a multidisciplinary approach. Methods: A 27-year-old woman was admitted to our hospital accusing headaches, impaired vision, nausea and motor deficit of the lower limbs developed over the past four months. Neuroimaging

revealed focal thickenings of the leptomeninges along the optic chiasm, brain stem and cerebellopontine angles which extended diffusely to the spinal cord and along the nerve roots of the filum terminale. A large intradural mass was observed at the C6-T1 level with smaller nodules clinging to the distal nerve roots. They appeared hyperintense in T1-weighted images. A partial resection of the spinal tumor was performed, revealing a dense, black mass. Results: Histopathological appearance featured nests of spindled and epithelioid, pigmented cells accompanied by numerous melanophages. Due to the heavy pigmentation, nuclear features were hard to assess although cytologic atypia was low. The presence of very rare mitotic figures as well as a Ki67 index of ~ 3% were indicative of an intermediate grade melanocytoma. Conclusion: The presence of an intermediate grade melanocytoma in the context of meningeal melanocytosis is worrisome. Rarely, this condition has been described in patients with neurocutaneous melanosis who are more at risk of developing malignant tumors. Our patient, however, did not show any skin lesions.

Keywords: Melanocytosis – melanocytoma – spinal tumor – case report

P189

Expression and gene regulation of calbindin in meningioma

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Introduction: Calbindin-D28k is a calcium-binding protein, mainly known to be expressed in the cerebellum and in the kidney. First discovered in 1966, the main function of calbindin seems to be buffering, whereas possible neuroprotective and cytoprotective effects are discussed. Calbindin has already been detected in a set of medulloblastoma, lung cancer and seems to be deregulated in subgroups of meningioma. Objectives: In this study, the primary target is to determine if calbindin is expressed in meningioma compared to their cells of origin, the meningeal cells of the arachnoidea mater, and to examine if expression goes along with a specific DNA methylation pattern. Methods: Therefore, immunohistochemistry of 76 meningioma and 10 pacchionic granulation samples with one monoclonal and one polyclonal anti-calbindin antibody was performed. A qPCR approach will compare RNA levels of the Calb1 gene to those of the RPL37A reference gene. Also, epigenetic modification of the Calb1 locus will be analyzed by bisulfite sequencing, to evaluate if a specific methylation pattern of calbindin expressing meningioma can be detected. Results: From the 76 meningioma samples, 17 (22%) showed positivity with the monoclonal and 23 (30%) with the polyclonal anti-calbindin antibody, in contrast to meningeal cells, showing no positivity. qPCR as well as methylation analysis are ongoing. Conclusion: The results confirm that Calbindin-D28k is overexpressed in a subset of meningiomas, throughout different histological subtypes, possibly indicating an own subclass of meningioma. This fuels the suspicion that calbindin might play a role in meningioma or their tumorigenesis with its specific function still unknown.

Keywords: Calbindin – meningioma – gene expression – gene regulation

P190

An undergoing tale of two techniques: digital slide analysis versus pathologists visual scoring in interpreting Ki-67 index in meningiomas

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Introduction: Meningiomas, both benign and malignant, represent up to 40% of primary intracranial neoplasms. Their prognosis cannot be reliably predicted because of the capricious behavior of meningiomas so different methods are still being investigated to overcome this debate. Purpose: The aim of this study is to evaluate whether digital image analysis (DIA) measured Ki-67 labeling index is prior to pathologists visual scoring (PVS) to estimate WHO grade and prognosis in meningiomas. Methods: Ki-67 expressions in hotspot areas of 152 adult meningioma cases (129 grade 1, 23 grade 2) were evaluated on the digitized slides with a commercial DIA program. The results were compared to PVS, WHO grade, histopathologic features and tumor recurrence. Results: DIA derived Ki-67 labeling index was both strongly correlated with PVS derived index ($p < 0.001$), and WHO grade ($p < 0.001$). ROC analysis showed a cut-off point of 9.1% for Ki-67 index for grade 2 meningiomas with a 0,78 sensitivity and 0,79 specificity. Furthermore, a cut-off point of 6.8% Ki-67 index was found for recurrent meningiomas with a 0.83 sensitivity and 0.64 specificity. Brain invasion did not show any correlation with Ki-67 index in WHO grade 2 meningiomas. When minor histopathologic criteria were compared with Ki-67 index in WHO grade 1 tumors, only presence of necrosis displayed significant correlation ($p = 0.026$). Conclusion: Ki-67 is found to be a reliable independent variable of grade and recurrence in meningiomas in our preliminary study. Also DIA, displaying strong correlation with PVS, seems to efficiently label Ki-67. It is imperative to do further research to define accuracy of our findings.

Keywords: Meningioma – Ki-67 – digital image analysis

P190Z

Predictive immunohistochemical markers in meningiomas

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Introduction: Meningiomas are one of the most frequent intracranial tumors. Although tumor recurrence is an important and not infrequent event in meningiomas, predictive immunohistochemical markers that could support the routine pathologic work-up have not been identified yet. Reviewing the literature has revealed the potentially predictive makers in meningiomas: p53, Ki-67 (Mib-1) and progesterone receptor (PR). Purpose: The aim of this study was to address a prognostic immunohistochemical panel by systematic retrospective analysis of surgically completely resected meningiomas with and without recurrence, including tumor samples from patients who underwent repeat surgeries. Methods: 114 surgical specimens of 70 meningioma patients (16 male and 54 female) in a 16 year interval have been studied. On Mib1, PR, and p53 immunostained sections, the percentage of labelled tumor cells, the staining intensity and the multiplied values of these parameters (the histoscore) was calculated. Results were investigated by Kruskal-Wallis H-test, Mann-Whitney U-test and Wilcoxon signed ranks tests. Results: Our results confirmed previous findings that the WHO grade is directly proportional to Mib1 and p53 and is inversely proportional to the PR immunostain. We have demonstrated that Mib1 and p53 have a significant correlation with and predictive value of relapse/recurrence irrespective of the histological subtype of the same WHO grade. Mib1 showed a significant correlation with the rate of progression (based on the propagation of WHO grades). Conclusion: The immunohistochemical panel of PR, p53, Mib1 in parallel with applying standard diagnostic criteria based on Hematoxylin & Eosin stained sections is sufficient and reliable to predict meningioma recurrence in surgically completely resected tumors.

Keywords: Meningioma – brain tumors

P190Z.1

Immunohistochemical correlates of *TP53* somatic mutations in brain tumors

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Introduction: Alterations in the TP53 gene can be observed in nearly half of the human cancers and are common in brain tumors. Despite controversy on the correlation between p53 accumulation and TP53 mutational status, immunohistochemical (IHC) detection of overexpressed protein is used as a surrogate tool of mutation analysis. Objectives: The aim of our study was to characterize IHC expression features of TP53 somatic mutations and define their frequency in human cancers, particularly in brain tumors. Materials and methods: A large-scale database analysis was conducted in the IARC TP53 Database (R17). Altogether, 7878 mutations with IHC features were retrieved representing 60 distinct tumor sites. Results and conclusion: Our study demonstrates that p53 immunopositivity largely correlates with *TP53* mutational status in cancer. We observed, that all brain tumor specific *TP53* mutations showed detectable levels of p53 immunoreactivity. However, an increased likelihood of false negative IHC associated with rare nonsense mutations was observed in other tumor sites (breast, colorectum, head & neck, lung, bladder and skin). Our bioinformatic findings indicate that p53 IHC – a routinely used methodology in diagnostic (neuro)pathology – has good sensitivity and specificity regarding the presence or absence of *TP53* mutations in brain tumors.

Keywords: TP53 – p53 – tumors

P190Z.2

Intracranial mesenchymal chondrosarcoma: a rare histopathological entity

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Introduction: Mesenchymal chondrosarcoma (MCS) is a rare malignant tumor, representing 2 to 10% of all chondrosarcomas. Histologically, MCS has a typical biphasic pattern consisting of mesenchymal cells with interspersed islands of hyaline cartilage. Intracranial MCS is particularly rare and is associated with a high mortality rate. The treatment of this tumor is still subject of debate, however wide resection followed by adjuvant therapy with chemo-radiotherapy appears to be the most effective strategy. Clinical case: 67-year-old woman with otherwise unremarkable past medical history, developed right hemicrania and progressive left-sided weakness. On neurological examination the patient presented with central left facial paresis and left hemiparesis (MRC 3/5).

Neuroimaging revealed a 56 mm right parieto-temporal cystic lesion with solid component, surrounded by edema, causing mass effect. The patient underwent urgent neurosurgery, with complete macroscopic removal of the lesion. Systemic study revealed multiple hepatic lesions, as well as irregular enhancement of uterine cervix requiring further investigation. On neuropathological evaluation the tumor presented lobules of malignant cartilaginous tissue, separated by areas with round cells containing prominent nuclei surrounded by fusiform pleomorphic cells. Genetic analysis using FISH identified HEY1-NCOA2 fusion detected in 30% of nuclei, favoring a diagnosis of mesenchymal chondrosarcoma. Two months later the patient presented worsening of deficits and headache suggesting intracranial hypertension with neuroimaging confirming a relapse of the tumor. Conclusion: This case highlights a rare tumor with distinct neuropathological features and atypical location and age of presentation. Recent molecular characterization of this entity may provide clues about its pathogenesis in the future.

Keywords: Chondrosarcomas –mesenchymal chondrosarcoma –HEY1-NCOA2 fusion

Targeted therapy in neuro-oncology

P191

Valproic acid modifies total DNA methylation level and attenuates temozolomide effect in glioblastoma cell lines

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Introduction: Valproic acid (VPA) is used for the treatment of epileptic events in the course of glioblastoma. There is also some evidence that it improves the clinical outcome in those patients. Temozolomide (TMZ) is a gold standard chemotherapeutic in glioblastoma. Objective: The aim of that project is to show the effects of VPA administration, alone and in combination with TMZ, on the total DNA methylation level. Methods: Using the nucleotide post-labeling method, we analyzed the total amount of 5-methylcytosine (m5C), the main DNA epigenetic mark, in DNA of glioblastoma (T98G, U118, U138), cancer (HeLa) and normal (HaCaT) cell lines treated with VPA, and a combination of VPA and with TMZ. Results: We observed dose-dependent changes in the total DNA methylation in neoplastic cell lines and the lack of such effect in a normal cell line. VPA at high concentration (250 – 500 μM) increased the m5C contents in DNA, even in a short time. However, the exposition of glioblastoma cells to the combination of VPA and TMZ caused an adverse synergistic effect resulting in DNA demethylation. Conclusion: Total DNA methylation changes in glioma cell lines under VPA treatment suggest the new mechanism of that drug action and promote clinical implications for adjusting VPA and TMZ therapy in glioblastoma patients.

Keywords: Valproic acid – temozolomide – DNA methylation – 5-methylcytosine – glioblastoma

P192/SY 3.4

Somatostatin receptor-targeted radiopeptide therapy in treatment-refractory meningioma

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Background: Somatostatin receptor (SSTR)-targeted peptide receptor radionuclide therapy (PRRT) represents a promising approach for treatment-refractory meningiomas. Objectives: To investigate the effect of SSTR-targeted PRRT on progression-free and overall survival in treatment-refractory meningioma patients. Methods: We performed an individual patient data (IPD) meta-analysis including all published meningioma patients treated with SSTR-targeted PRRT. Main outcomes were toxicity, response to treatment, progression-free survival (PFS), and overall survival (OS). We applied the Kaplan-Meier method to estimate survival probabilities and report incidence rates per 100 person-years. We applied Cox proportional hazards models to determine the effect of covariates. Results: We screened 537 papers, and identified six eli-

gible cohort studies. We included a total of 111 patients with treatment-refractory meningioma who received SSTR-targeted PRRT. Disease control was achieved in 63% of patients. Six-month PFS was 94%, 48% and 0% for WHO (World Health Organization)-I, -II, & -III, respectively. The risk of disease progression decreased by 13% per 1000 MBq increase in the total applied activity. One-year OS was 88%, 71%, and 52% for WHO-I, -II & -III, respectively. The risk of death decreased by 17% per 1000-MBq increase of the total applied activity. Main side effects comprised transient hematotoxicities such as anemia in 22%, leukopenia in 13%, lymphocytopenia in 24%, and thrombocytopenia in 17% of patients. Conclusion: This IPD meta-analysis represents the most comprehensive analysis of benefits and adverse events of SSTR-targeted PRRT for treatment-refractory meningioma. The treatment was well tolerated, achieved disease control in most cases, and showed promising PFS and OS.

Keywords: Meningioma – targeted – progressive – PRRT – somatostatin

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PIK3CA mutant metastatic breast cancer presenting as a meningeal syndrome: case report

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Introduction: A 51-year-old woman presented complaining of headaches, temperature and nausea. A meningeal syndrome was diagnosed, imaging and cerebrospinal fluid (CSF) studies were performed. Objective: To present a case report of a singular presentation of a solid tumor with durable response to targeted therapy. Methods: CT scan showed multiple lytic bone lesions and meningeal carcinomatosis without a known primary solid tumor. CSF contained clusters of malignant epithelial cells, positive to estrogen receptors (ER). Breast cancer was suspected. Posterior specific examinations revealed a primary infiltrating lobular carcinoma. Inmmunophenotype was luminal B. PIK3CA mutation was found. Results: The patient started systemic therapy with letrozole in combination with palbociclib. She has achieved and maintained durable response for 32 months. Neurologic symptoms disappeared soon after starting treatment. Conclusion: Meningeal syndrome is a rare clinical presentation for metastatic breast cancer. CSF analyses and mutational profiling allows delivering early targeted therapy. Great durable responses can be achieved for stage IV disease.

Keywords: PIK3CA – breast cancer – metastasis – meningeal syndrome

P194/SY 3.6

Nanocarrier using protoporfirin IX-loaded albumin nanoemulsion for image-guided glioblastoma therapies

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Introduction: Nanocarriers based on albumin are nontoxic, biocompatible and biodegradable particles that have been studied as new alternatives for drug and gene delivery system (DDS) in oncology. The most relevant characteristic of these protein-based DDS is the capability to cross the blood-brain barrier (BBB). Glioblastoma (GBM) remains one of the most lethal tumors due to the resistance of the standard therapy and its high infiltrative invasiveness into adjacent brain parenchyma, where the BBB is often closed. Methods: This study evaluated the in vitro action of an albumin nanoemulsion (HSANE), previously validate to cross effectively the BBB in animal models in our laboratory, using three different GBM cell lines (U87-MG, A-172 and KNS-42). After characterization of its physical-chemical properties (average diameter, polydispersion index and zeta potential), HSANE particles were submitted to spectroscopic analyzes, cytotoxicity assays and PPIX internalization by confocal fluorescence microscopy. HSANE particles exhibited a mean size of 270 nm, low polydispersity (0.3), positive zeta potential (+40 mV) and effective incorporation of the compound (Protoporfirin IX) at the concentration of 0.9 $mg \times mL^{-1}$. The cell viability was assessed after 6 hours of PPIX-HSANE (PPIX concentrations 0.5, 1.0 and 3.0 μM) incubation, with cell viability around 80 to 100% considered non-toxic to toxic cells. Results: The PPIX remained in the cytoplasmic region of the cells after administration confirmed by fluorescence microscopy studies. These data represent a proof of concept that can be explored in the design of this multifunctional DDS based on Albumin Nanoparticles to achieve an efficient drug delivery in GBMs.

Keywords: Glioblastoma therapy – albumin nanoemulsion – drug delivery system – blood-brain barrier

Storage disorders

P195

Findings at neuroautopsy in a patient with Hurler-Scheie syndrome

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Background: Hurler-Scheie Syndrome (HSS) is a rare lysosomal storage disease classified as an intermediate type 1 mucopolysaccharidosis. A mutation in the IDUA gene causes a deficiency in the alpha-L-iduronidase enzyme. This leads to accumulation within lysozymes of dermatan and heparan sulfate causing characteristic dysmorphic features, heart disease, and neurologic manifestations. Case Findings: This 28-year-old man presented with a history of HSS characterized by spinal stenosis, heart disease, pulmonary hypertension, recurrent pulmonary infections, dystosis multiplex, and hearing loss with a 2-week history of worsening dyspnea and edema progressing to hemoptysis. Spinal MRI demonstrated dysmorphic cervical, thoracic, and lumbar vertebral bodies as well as both possible mega cisterna magna and encephalomalacia, and dural ectasia. He rapidly deteriorated with signs of multiorgan failure and on day 2 of hospitalization he developed acute mental status changes, pulseless electrical activity, and respiratory distress, expiring shortly thereafter. Findings at general autopsy included dysmorphic features, cellular and extracellular mucopolysaccharide throughout multiple organs, and acute on chronic pulmonary thromboemboli. Neuroautopsy revealed megalencephaly, diffuse ventriculomegaly secondary to hydrocephalus and absence of the septum pellucidum, severe cerebellar chronic neuronal loss and gliosis with grossly thickened folia, dilated white matter perivascular spaces with surrounding loose fibrous tissue, abnormally angled hippocampi, a focus of neurophagia in the midbrain, and neuronal loss in the neocortices. Cause of death was PEA cardiac arrest most likely due to mucopolysaccharidosis and acute on chronic pulmonary emboli. Conclusion: HSS is rare and all cases are important to publish to increase the literature on disease extent and outcomes.

Keywords: Hurler-Scheie Syndrome – mucopolysaccharidosis – congenital and genetic diseases

P196

Tau pathology in Niemann-Pick type C follows Braak's Alzheimer's disease staging – insights from an 18-year-old patient autopsy

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Introduction: Neurofibrillary tangles (NFT) similar to those in Alzheimer's disease (AD) are a common feature in Niemann-Pick type C (NPC), raising interest in the role of impaired cholesterol metabolism in their formation in both diseases. Objectives: To describe the topographic relation between tau pathology and the neuronal storage severity. Methods: Clinical and neuropathological description. Results: An 18-year-old female, with learning difficulties noticed at 8-years-old, started gait ataxia 6 years later, followed by dysphagia, dysarthria, and urinary incontinence. In the first visit (17-years-old), she had supranuclear vertical gaze palsy, dysphagia, cerebellar dysarthria, and appendicular ataxia. Exome sequencing analysis revealed two compound heterozygous disease-causing variants in the NPC1 gene and started treatment (miglustat). Two months later was admitted with a subacute encephalopathy and severe hyperkinetic movement disorder. Diagnostic workup was negative and died one month later (pneumonia). The neuropathology study showed numerous swollen neurons distributed throughout the neuroaxis and NFT in the hippocampus, entorhinal region, inferior temporal gyrus, thalamic anterior nucleus, amygdala and Meynert nucleus, reminiscent of Braak stage III. In these regions most of the swollen storage neurons had NFT. There was no neuritic plaques or Aβ deposition. Additional perivascular inflammation and microglial nodules were seen raising the question of additional auto-immune encephalitis. Conclusion: Previous work reported that in cases older than 25, the number of NFT in the hippocampus increase exponentially similar to classic stage III AD. We further extended this relation into other areas, reinforcing anatomical susceptibility and progression pattern of tau pathology in an AD-like pattern.

Keywords: Niemann-Pick – Alzheimer's disease – tau – Braak stage

P197

Mitochondrial dysfunction associated with *CLN1* knock-out in SH-SY5Y neuronal-like cells

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Introduction: Mitochondrial dysfunction can occur in different human pathological conditions, in-

cluding lysosomal storage diseases affecting the brain. Neuronal Ceroid Lipofuscinoses (NCL) are progressive neurodegenerative disorders of childhood with fatal outcomes, characterized by endo-lysosomal storage and diffuse neuronal death. CLN1 disease (OMIM #256730) is an early childhood onset NCL associated with mutations in CLN1/PPT1, a hydrolytic enzyme involved in the removal of palmitate from S-acylated proteins within lysosomes as well as in other cellular compartments. Purpose: The widespread loss of neurons in CLN1 patients led us to investigate the putative role of mitochondria in the cell degeneration process, utilizing a neuronal-like cellular model of the disease. Methods: We utilized CRISPR/Cas9 genome editing on SH-SY5Y cells to generate a CLN1-KO neuronal-like model. Morphological and biochemical tools were used to analyze mitochondrial reticulum and functioning; bioenergetic features were investigated through enzymatic activity assays as well as by micro-oxygraphy. Results: Unbalanced mitochondrial membrane polarization state was evident in CLN1-KO cells. Micro-oxygraphy indicated a defective oxygen consumption rate (OCR) and a concurrent glycolytic shift, suggesting a compromised mitochondrial respiration. Supporting evidence of metabolic alterations were also given by bioinformatic findings after RNA-seq transcriptomic analysis. Moreover, maturation of axonal-like processes was defective. Conclusion: Loss of function of CLN1/PPT1 impairs some aspects of the mitochondrial functioning, including membrane polarization state and OCR, which may secondarily affect energy-demanding cellular functions. These findings are in accordance with our previous findings on patients' fibroblast cells. How defective PPT1 activity and lysosomal storage hamper bioenergetics of neurons may deserve further inquiries.

Keywords: Neuronal ceroid lipofuscinosis – mitochondrial alterations – micro-oxygraphy – rna-seq transcriptomic analysis

Neuromuscular disorders

P198

Acquired TTR amyloid neuropathy – insights from a nerve biopsy cohort study

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Background: Pathophysiology of acquired TTR amyloidosis (acqATTR) developing after domino liver transplant (DLT) remains poorly understood. This form of neuropathy has been shown to bear few clinical differences compared to hereditary TTR amyloidosis (hATTR). Objectives: To describe a clinicopathologic cohort of acqATTR patients focusing on nerve biopsy studies. Methods: We compared neuropathy characteristics between acqATTR with hATTR patients with similar age at biopsy and controls. Morphometric studies, amyloid deposits characterization and fundamental neuropathological data was obtained from sural nerve biopsies. Symptoms onset and progression was also reviewed. Results: 13 patients (11M:2F) with acqATTR amyloidosis were identified with a mean DLT age at 53.5 years, a mean age of neuropathy onset at 60.5 years and a DLT-to-neuropathy interval of 7 years (range 4 – 13 years). Hereditary TTR patients (24 cases) had a worse polyneuropathy disability score at biopsy comparing to acqATTR patients with similar disease duration ($p = 0.042$). Controlling for disease severity, both patients groups had lower total myelinated fiber densities compared to controls (12 cases), but acqATTR patients were shown to differ from controls only for small myelinated fiber densities ($p = 0.039$). Amyloid deposition was detected in 9 acqATTR and 8 hATTR patients (75% versus 57%, $p = 0.34$). Discussion: Despite a similar interval between disease onset and nerve biopsy, hATTR patients presented higher clinical severity compared to acqATTR patients. Similarly to what has been described for hTTR, small myelinated fibers are affected earlier at the onset of neuropathy. Additional mechanisms must be sought to elucidate further clinical and pathological differences between the two groups.

Keywords: Acquired TTR amyloidosis – domino liver transplant – nerve biopsy – morphometry – small fiber neuropathy

P199/SY 8.4

NanoString technology distinguishes anti-TIF-1γ$^+$ from anti-Mi-2$^+$ dermatomyositis patients

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Background: Dermatomyositis (DM) is a systemic idiopathic inflammatory disease affecting skeletal muscle and skin, clinically characterized by symmetrical proximal muscle weakness and typical skin lesions. Myositis-specific autoantibodies (MSA) such as anti-TIF-1γ autoantibody strongly correlate with distinct clinical manifestations, prognosis and increased risk of

cancer-associated myositis (CAM). Objectives: To investigate alternative reliable methods to distinguish DM subgroups, further stratify DM patients and analyze the signaling pathways involved in CAM development. Methods: Evaluation of skeletal muscle biopsies from anti-TIF-1γ- and anti-Mi-2-associated DM patients by the NanoString technology, qPCR, immunohistochemical and immunofluorescent staining. Results: We demonstrate that the NanoString technology can be used as a very sensitive method to clearly differentiate these two clinically distinct DM subgroups. Using the nCounter Pan-Cancer Immune Profiling Panel™, we identified a set of significantly dysregulated genes in anti-TIF-1γ^{+} patient muscle biopsies including *VEGFA*, *DDX58*, *IFNB1*, *CCL5*, *IL12RB2,* and *CD84*. Investigation of type I IFN-regulated transcripts revealed a striking type I interferon signature in anti-Mi-2^{+} patient biopsies. Conclusion: Our results help to stratify both DM subgroups and predict, which DM patients require an intensified diagnostic procedure and might have a poorer outcome. Potentially, this could also have implications for the therapeutic approach.

Keywords: Dermatomyositis – myositis-specific antibody – NanoString – TIF-1γ

P200

Spinal encoding of motor deficits induced by a unilateral brain injury: analysis of hindlimb motor responses

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Introduction: Mechanisms of motor deficits secondary to stroke and traumatic brain injury remain poorly understood. Purpose: Here we demonstrate that hindlimb postural asymmetry in rats is induced by a unilateral injury of the hindlimb sensorimotor cortex, and characterize this phenomenon as a model of spinal neuroplasticity underlying asymmetric motor deficits. Methods: A unilateral brain injury was performed, followed by a complete spinal transection or a bilateral rhizotomy. Nociceptive withdrawal reflex stimulation was recorded using EMG technique. Result: After cortical lesion, the asymmetry was developed due to the contralesional hindlimb flexion and persisted after decerebration and complete spinal cord transection. The asymmetry induced by the right-side brain injury was not eliminated by bilateral lumbar dorsal rhizotomy. Pancuronium, a curare mimetic muscle relaxant, abolished the asymmetry suggesting its dependence on the efferent drive. A brain injury resulted in greater activation of the nociceptive withdrawal reflexes evoked by electrical stimulation and recorded with EMG technique on the contra- versus ipsilesional side. The unilateral brain injury modified expression of neuroplasticity genes and robustly impaired coordination of their expression within and between the ipsi- and contralesional halves of the lumbar spinal cord. Conclusion: Our data suggest that changes in the hindlimb posture and nociceptive withdrawal reflexes are encoded by neuroplastic processes in lumbar spinal circuits induced by a unilateral brain injury. The mechanism that is independent of afferent input may mediate asymmetric hindlimb motor responses and may be based on sustained muscle contractions which often occur in patients with central lesions and which are not evoked by afferent stimulation.

Keywords: Asymmetry – brain injury – neuroplasticity

P201

ACTA1-related myopathy with focal myofibrillar pathology and cytoplasmic bodies

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Introduction: Actinopathies are a clinically and histopathologically heterogeneous group of myopathies involving pathogenic variants in the skeletal muscle α-actin (*ACTA1*) gene, classically associated with nemaline myopathies. Objectives: To describe an *ACTA1*-related myopathy with mosaicism and peculiar focal pathology. Methods: Clinical, histopathological and genetic characterization. Results: An 8-year-old boy presented with suspected myopathy. Pregnancy was uneventful and no family history or consanguinity were reported. Intellectual development and motor milestones were unremarkable, despite a mild facial asymmetry noted at 9 months. Examination revealed a high-arched palate, lower right facial weakness, striking flexor and extensor cervical weakness, asymmetric left-predominant scapular atrophy and winging, and a mild feet dorsiflexion paralysis. CK levels were normal. A deltoid muscle biopsy showed mild global myopathic features, with multifocal areas of atrophic fibers with numerous internal nuclei, profound myofibrillar disorganization, reduced or absent oxidative staining and frequent cytoplasmic bodies. Type 1 fiber

predominance was observed but no cores or nemaline bodies were found. Immunohistochemistry revealed cytoplasmic dystrophin and desmin aggregates in fibers with myofibrillar pathology. NGS gene panel for congenital myopathies documented a novel heterozygous variant in *ACTA1:* NM_001100.3:c.461T > C (p.Val154Ala). This substitution was present in about 25% and 27% of the NGS reads, derived from muscle and blood DNA, respectively. This lower allele fraction variant was considered suggestive of somatic mosaicism. Discussion: Cytoplasmic bodies and myofibrillar pathology are rare in actinopathies, particularly in the setting of absent nemaline rods. We hypothesize that asymmetric clinical findings and multifocal pathology could be considered clues towards an underlying mosaic myopathy.

Keywords: Nemaline myopathy – ACTA1 – cytoplasmic bodies – myofibrillar – mosaicism

P202

Mitochondrial myopathy: a case report

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Introduction: Mitochondrial Myopathies are a heterogeneous group of diseases whose common anatomopathological substrate is anomalous mitochondrial proliferation. Symptoms such as predominantly proximal bilateral and symmetrical muscle weakness, pelvic tilt gait and characteristic difficulty in getting up are observed. Muscle biopsy with immunohistochemical study (IMH) is essential for the definitive diagnosis of neuromuscular diseases, particularly myopathies. Electron microscopy (EM) is important in investigation of pleochonial and megaconal mitochondrial alterations, typical of mitochondrial diseases, especially when other findings are inconclusive. Pathological abnormalities that can be found on histopathological examination are mitochondrial proliferation ("ragged-red") and deficiency of enzyme complexes that make up oxidative phosphorylation systems. Thus, the objective of present study is to report a case and perform a literature review. Case and findings: MJNV, 47-year-old male. Skeletal muscle biopsy showed mild and nonspecific muscle changes, with minimal variability in the diameter of skeletal muscle fibers and presence of fiber regeneration. Histochemical and special staining without alterations and IMH findings show normal protein positivity. EM showed mitochondria with signs of swelling, larger than usual size and ridges in concentric arrangement, suggestive of mitochondrial myopathy. Conclusion: The approach of mitochondrial diseases is encouraging and points to the identification, prevention and treatment of complications, although there is no treatment for genetic abnormality. Therefore, early diagnosis is essential and use of complementary diagnostic resources such as IMH and ME, associated with clinical evaluation and correct interpretation of signs and symptoms, is important. The diagnosis should be followed by genetic counseling for proper guidance of the individual and the family.

Keywords: Mitochondrial myopathies – muscle biopsy – electron microscopy

P203

Mitochondrial disorders in adults: histopathological and ultrastructural correlations in Polish patients with nuclear DNA mutations

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Background: Mitochondrial disorders result from impaired oxidative phosphorylation due to mutations in mitochondrial (mtDNA) and nuclear genome (nDNA). Objectives: Histopathological and ultrastructural assessment of muscle biopsy specimens from Polish adult patients with mitochondrial disorders and nDNA mutations. Methods: Clinical, electrophysiological and morphological assessment of thirteen adult Polish patients were done. The muscle specimens were assessed on light and electron microscopy. Genetic studies of mtDNA and nuclear encoded *POLG*, *TWNK* (*C10orf2),* and *RNASEH1* genes were performed. Results: Chronic progressive external ophthalmoplegia (CPEO) was identified in 4 patients, chronic progressive external ophthalmoplegia plus proximal myopathy (CPEO+) in 4, progressive external ophthalmoplegia and the central nervous system impairment classified as mitochondrial encephalomyopathy (ME) in 3, sensory ataxic neuropathy, dysarthria and ophthalmoparesis syndrome (SANDO) in 1 and myoclonic epilepsy in 1 patient. Histopathological assessment revealed primary myopathic features. Ragged-red fibers were detected in 11 biopsies. Ultrastructural analysis showed various changes. Genetic studies of mtDNA proved single or multiple mtDNA deletions in most cases in the muscle tissue. Mutations in *POLG* gene were confirmed in 3 CPEO patients, in 3 CPEO+, in one with ME, in one with myoclonic epilepsy and in one with SANDO syndrome. The analysis of the *TWNK* (*C10orf2)* gene proved the mutation in one CPEO and one CPEO+ patient. Assessment of *RNASEH1* gene revealed homozygous variant in two siblings with ME. Conclusion: Histopathological and ultrastructural assessment of muscle biopsy specimens is essential for mitochondrial disorders. Genetic studies of both mtDNA and nDNA are necessary for final diagnosis, genetic counseling and treatment.

Keywords: Mitochondrial disorders – muscle biopsy – mitochondrial DNA

P204

Muscle biopsy role in identification of McArdle disease prior to genetic test

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Introduction: McArdle disease (McA) is an inborn error of glycogen metabolism, characterized by deficiency in glycogen myophosphorylase, broad clinical spectrum, and often misdiagnosis. Muscle biopsy reveals the presence of subsarcolemmal glycogen vacuoles and deficiency of myophosphorylase in histochemistry. Methods: Retrospective analysis of cohort of patients with muscle biopsy suggestive of McA from a single neuropathology center between January 2010 and December 2020. Histochemistry for myophosphorylase is routinely made in our laboratory. Available clinical features and complementary studies were analyzed. Results: Fourteen patients were included, 64.3% were female. Except for one case (age 14) in which McA was considered early on, in all cases, the initial diagnosis was other than McA. The muscle biopsy was ordered with clinical suspicion of autoimmune/inflammatory myositis (n = 7, 50%), toxic myopathy (n = 3, 21.4%), isolated episode of rhabdomyolysis and persisted hyperCKemia (n = 3), metabolic myopathy (n = 2, 14.3%), congenital myopathy (n = 1, 7.1%). Mean age at the beginning of first symptoms was 20.9 (1 – 60), mean age at diagnosis was 37.6 years (10 – 71), with an average of 16.7 years to diagnosis. Most patients (78.6%) had intolerance to exercise, followed by myalgias (71.4%), muscle weakness (57.1%), and second wind phenomenon (14.3%). All patients had elevated CK levels (maximum CK 4097-177 000). Five patients had myopathic patterns on electromyography. Conclusion: Genetic testing performed early-on in high clinical suspicion cases is the current gold-standard for McA. However, McA presents marked clinical heterogeneity, including atypical and delayed-onset symptoms. In these patients, muscle biopsy with routine histochemistry for myophosphorylase proved to be essential for the diagnosis.

Keywords: McArdle disease– muscle biopsy – myophosphorylase

P206/WS 2.7

Automated large-scale scanning transmission electron microscopy of myopathies with structural abnormalities

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Introduction: Distinct ultrastructural findings may be encountered in a large variety of neuromuscular disorders. Here, electron microscopy (EM) represents an essential tool in diagnostic and research settings, especially in context of new classifications. However, manual imaging using a transmission electron microscope (TEM) is time-consuming and biased. Alternatively, large-scale digitization by automated recording and stitching of overlapping images allows a more precise examination. Limitations are, however, slow imaging speed and small field dimensions. Purpose: We aimed at an improved large-scale digitization to achieve automated recording of multiple sections in a reasonable time span, while maintaining image quality. Methods: A Zeiss Gemini 300 field-emission scanning electron microscope (FE-SEM), equipped with a scanning transmission electron microscopy (STEM) detector, was used. Imaging parameters such as dwell time, pixel size and tile size were varied to accelerate acquisition and maintain image quality of relevant structures. Results were compared to TEM-imaging. Results: STEM-imaging provided increased image fields of 10,240 pixels per side. Using a pixel size of 9 nm, large images of 92 µm per side were recorded, providing a sufficient amount of structural details in overlapping areas for later stitching, while TEM-imaging provided 2048 pixels per side, thus recording many "empty" images. 10× faster imaging was achieved, enabling digitization of whole 2×1 mm sections in about 6 hours, while identifying filaments and organelles. Conclusion: We demonstrate improved large-scale digitization of ultrastructural abnormalities using STEM-imaging, allowing automated digitization of 12 sections in 3 days. The resulting datasets provide an ideal basis for fast and in-depth analysis of ultrastructural abnormalities.

Keywords: Electron microscopy – large-scale digitization – myopathology – nanotomy

Other

P207/SY 1.4

Regional proteomic mapping of the human vanishing white matter brain

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Introduction: Vanishing white matter (VWM) is a fatal leukodystrophy caused by mutations in genes encoding the eukaryotic translation initiation factor 2B, ubiquitously expressed and crucial for translation. In VWM, the brain white matter is selectively affected with a spatial feature in which different regions are selectively vulnerable and develop pathology at different time points during disease progression. Methods: To delineate what drives this selective vulnerability and identify therapeutic candidates, we analyzed protein expressions within different gray and white matter regions of human control and VWM brains. Four regions (cortex and frontal lobe, cerebellar, and pons white matter) were laser capture microdissected and subjected to high-resolution mass spectrometry-based proteomics. We quantified over 2800 proteins across control and VWM brain tissues. Results: Our analysis identified proteins altered in VWM associated with processes including synaptic activity, transcription and translation, extracellular matrix and cytoskeletal organization, and energy metabolism. Importantly, we found region-specific protein changes. Some proteins are affected in the VWM white matter regardless of regional differences in disease severity. Other proteins are associated with chronic degeneration, and only found altered in the more severely affected VWM white matter areas. Notably, we also provide evidence for gray matter pathology in VWM. The cortex, rather than being spared, displays a distinct proteome from control and other regions in VWM, reflecting a disease feature that has been overlooked so far. Taken together, this study provides insights into the human VWM brain proteome along with the identification of target candidates to abate this disease.

Keywords: Vanishing white matter – proteomics – regional vulnerability

P208

Hypothalamitis: a novel autoimmune endocrine disease. A literature review and case report

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Introduction: Autoimmune hypothalamitis is described as a rare autoimmune condition in which the hypothalamus is infiltrated by lymphocytes and plasma cells. Although several immunohistochemical biomarkers were cited to this rare entity in the literature, none was specific for it. Objective: We report a case of an isolated autoimmune hypothalamitis with partial empty sella syndrome (ESS), central diabetes insipidus (CDI), and hyperprolactinemia and compare our results with the information in the literature. A 35 years-old woman diagnosed with menopause by a third-party medical center due to oligomenorrhea presented to our hospital with hyperprolactinemia. Clinically she was diagnosed as having CDI and panhypopituitarism. Methods: MRI scan revealed an intraventricular mass obliterating the infundibular recess on the third ventricle floor. Histopathology and immunohistochemistry panels were consistent with a lymphocytic hypothalamitis. Double immunofluorescence technique (DIF) was used to investigate antibodies to arginine vasopressin (AVP) secreting cells (AVPcAb), and antipituitary antibodies (APA). Results: Immunohistochemical analysis ruled out the diagnoses, i.e. PCD, LCH, RDD, Ig-G4RD. The presence of AVPcAbs was identified by DIF and targeting AVP-secreting cells but no evidence of APA was found. Conclusively, it was consistent with autoimmune hypothalamitis. Conclusion: In the final analysis, diagnosis of suprasellar masses based upon immunohistochemistry should be appended with serologic markers, i.e. APA, and AHA. This is the first observation of autoimmune hypothalamic involvement with CDI, partial ESS, AHA antibodies, and hypopituitarism. The challenge in its diagnosis resides in a limited number of cases reported.

Keywords: Autoimmune hypothalamitis – anti-hypothalamus antibodies (AHA) – central diabetes insipidus – isolated hypothalamitis – lymphocytic hypothalamitis

P209

Application of the new "Digital Brain" platform of the archive of human brains in scientific research on Alzheimer's disease (AD)

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Introduction: Poland has one of the largest collections of patients' brains, containing examples of many neurological disorders, archived in the Institute of Psychiatry and Neurology in Warsaw. The tissue collection is transferred onto a "Digital Brain" platform, enabling wider access to catalogue containing: brain slices, paraffin-embedded samples, slides and clinical and neuropathological diagnoses. Methods: One of the examples of using these resources is scientific research, aimed at search-

ing for risk factors of Alzheimer's disease (AD) and developing new methods for early diagnosis. Using diagnostic material, collected in the archive, allows translation of results obtained in preclinical studies, conducted on experimental animals, into clinical cases and validates the newly developed animal models. An example of such usage is our project, which aims to search for molecular mechanisms and assess lifestyle-related factors as risk factors for the AD development. Western diet (WD) was used in this project as a factor, triggering the cascade of events leading to Alzheimer's degeneration by inducing metabolic syndrome in experimental mice. Results: The results confirmed that the WD led to the development of neuropathological changes in the mice brains, comparable to those observed in the brains of AD patients. Conclusion: "Digital Brain" platform can be used not only for scientific research but it may also help to expand knowledge, regarding the diseases pathogenesis and progression, support the medical programs and assist the development of new treatment methods for patients with mental, neurological and neuro-oncological disorders. Projects financed by 1) Digital Poland Project Centre, No. POPC.02.03.01-00-0042/18-00, 2) National Science Centre No. 2014/15/D/NZ4/04361.

Keywords: Digital Brain – biobanking – Alzheimer's disease

P210/SY 1.5

Volume and cell number of the hippocampus in depression, schizophrenia, and suicide subjects

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Introduction: Many in vivo imaging studies report reduced volume of the hippocampus in major depressive disorder and schizophrenia. In our study, we investigate if depression, schizophrenia or suicide is associated with reduced volume of the hippocampal formation and/or changes in the number of neurons and/or glial cells in the hippocampus. Methods: We base our study on postmortem brain samples from 10 subjects with schizophrenia, 8 subjects with major depression, 11 suicide subjects with a history of depressive disorder, and 10 control subjects with no history of psychiatric or neurological diseases. We use light microscopy and design-unbiased stereological Methods: The Cavalieri estimator is used to estimate the volume of hippocampus and its subregions, and the optical fractionator method is used to estimate the total number of neurons and glial cells in the individual cell layers in four main regions of hippocampus: the granular cell layer, hilus, CA2/3, and CA1. Results: We found 20% to 35% reductions of the volume and the number of neurons and glial cells in the subjects with depression or schizophrenia relative to control subjects across all hippocampal regions. In suicide subjects, we found increased neuron number in CA2/3 subregion. The volumes and cell numbers are reduced similarly in depressed and schizophrenia subjects relative to control subjects across all hippocampal regions. Conclusion: Thus, our findings imply that the hippocampus may be a common site of pathophysiology in depression and schizophrenia. In our study, suicide subjects have a different neurobiology in hippocampus compared to subjects dying with major depression without suicide.

Keywords: Stereology – hippocampus – depression – schizophrenia – suicide

P211

Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia, phenotypic and clinical heterogeneity

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Introduction: Adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) is a rare autosomal dominant neurodegenerative disorder with distinctive histopathologic features. ALSP typically affects young adults and exhibits a broad spectrum of clinical manifestations that vary from patient to patient, including cognitive impairment, psychiatric symptoms, movement disorders and speech symptoms. Objectives: The present study aims to contribute to a greater understanding of ALSP, highlighting the degree of variability of its phenotypic and clinical expression. Methods: We report three ALSP cases diagnosed at brain autopsy in our Brain Tissue Bank (Banco de Tejidos de la Fundación CIEN, Madrid, Spain). Results: Age of onset ranged from 49 to 66 years, with 4 to 6 years of disease progression until death. The dominant symptoms were variable among the three patients, characterized by frontal lobe dysfunction with parkinsonism, gait disturbance and cognitive decline respectively. Microscopically all cases revealed the pathologic hallmark of ALSP, showing extensive myelin and axonal loss, pigmented glia and axonal spheroids with variable intensity and anatomical distribution; in addition one case displayed striking microvascular calcification and another case presented an atypical discontinu-

ous pattern of hippocampal sclerosis. Conclusion: ALSP is an underdiagnosed entity, further knowledge of its clinical heterogeneity is crucial to increase diagnostic accuracy and suggest genetic testing on affected patients. Phenotypic neuropathological variations contribute to clinical presentation and should be considered.

Keywords: Leukoencephalopathy axonal spheroids

P212

The potential for digitalization of frozen sections – results from a workflow analysis

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Introduction: Frozen sections help to determine tissue status and support neurosurgeons during tumor resections. Samples are sent to the neuropathologist for analysis, who delivers results via telephone to assist intraoperative decision-making. Recently, new devices, using confocal endomicroscopy to digitally visualize tissue structures on a monitor, have entered the market. Objectives: To analyze the current workflow of tumor resection and compare it to a process using confocal endomicroscopy. Methods: An exemplary process for tumor resection with frozen sections (current) and a digitized process (ideal) have been modelled using Business Process Model Notation (BPMN) 2.0. BPMN is a standard for graphical process representation. Results: Two institutions are involved, the clinic and the neuropathology department. Currently, seven people (neurosurgeon, surgery assistant, neuropathology receptionist, neuropathology secretary, lab technician, Jr & Sr neuropathologist) are required for a total number of 42 tasks. The ideal process employs three people for 24 tasks (–43%). The digital biopsy device is operated in the clinic, therefore the number of tasks slightly increased compared to the current process (from 11 to 14, +27%). Workload in the neuropathology is reduced due to the elimination of administrative and laboratory tasks (from 31 to 10, –68%). Conclusion: Using digital biopsy, the number of samples per patient is not limited. The digital connection and continuous communication between neurosurgeon and neuropathologist offer potential for real-time diagnostic and joint decision-making. Administrative and laboratory workload could be significantly reduced. Consequently, digital biopsy can potentially shorten surgery duration, improve patient outcomes and relieve workload for the neuropathology department.

Keywords: Digital biopsy – frozen section – workflow analysis

P213

Recurrent acute ischemic "stroke" from fibrocartilaginous cerebral emboli – A case report

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Background: Fibrocartilaginous embolism has rarely been reported as a cause for acute ischemia of the spinal cord. It is presumed to be due to retrograde embolization of nucleus pulposus material from intervertebral disc. A single case report has previously documented fibrocartilaginous embolism to the middle cerebral artery demonstrated on post mortem examination. We report a case of paradoxical fibrocartilaginous cerebral embolism in a patient via a Patent Foramen Ovale (PFO). Case report: A 48-year-old man presented with episodes of worsening headache and intermittent neurological symptoms including dizziness, confusion, visual defect and sensorimotor disturbances involving both hands and upper limbs. Magnetic resonance imaging (MRI) showed multifocal acute and chronic infarction. Investigations ruled out infective, coagulative and autoimmune causes. Despite anticoagulation therapy, recurrent acute ischemic episodes confirmed on MRI continued. Brain biopsy performed.to exclude vasculitis revealed evidence of multifocal micro infarction within the cerebral parenchyma. A fragment of fibrocartilage was visualized in the lumen of a leptomeningeal artery whilst some blood vessels showed luminal debris and foam cells. Subsequent echocardiogram revealed a PFO associated with right to left shunt. An MRI of the spine performed to identify the source of paradoxical fibrocartilaginous embolism showed only a disc bulge at the L5-S1 level. The patient has remained stable with no new lesions identified on serial MRI imaging since closure of PFO and follow up period of l year. Conclusion: Paradoxical fibrocartilaginous cerebral embolism via a PFO is a rare finding in patients who present with recurrent acute ischemic "stroke" like episodes.

Keywords: Fibrocartilaginous emboli – ischemic stroke

P214

Importance of molecular diagnosis of central nervous system tumors and legal implications in Brazil

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Introduction: Molecular neuropathology has undergone a revolution because of development of high throughput techniques so that biomarkers play a critical role in diagnosis and prognosis. Some patients are unable to afford financial costs of molecular testing, qualitatively reducing the data essential for accurate diagnosis. Such reduction may make the pathologist's report more vulnerable to distorted interpretations. Objectives and Methods: Importance of diagnosis and problems in countries where tests are not available by search for actual datas about the subject in specialized websites. Traditionally, diffuse gliomas were classified into astrocitoma, oligodendroglioma and oligoastrocytoma. But with molecular pathology, some terms are being abandoned while others have become more specific. For example, the diagnosis between diffuse astrocytoma and oligodendroglioma, which can now be defined by molecular tests. Advances in development of immunohistochemical markers can facilitate more accurate diagnoses, which is more affordable financially. In Brazil, civil liability of doctors is subjective. The item 14 of the Consumer Protection Code determines that liability of liberal professionals is defined by proof of guilt in their performance. As a rule, doctors are responsible for treatment, not cure. In specific situations they account for results. The pathologist, in turn, when issuing reports, responds subjectively in cases of error, but submits an obligation of result as to their delivery. If, however, harm caused to a patient was due to misinterpretation of the report, liability will be of the doctor who proposed the harmful treatment or did not indicate appropriate treatment. Conclusion: Thus, it is necessary to expand access to molecular tests.

Keywords: Molecular pathology – tumors – legal implications

P215

BRAIN UK: accessing NHS diagnostic archives for neuroscience research

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Introduction: Human tissue can be difficult for researchers to access, with time consuming, challenging legal and ethical considerations. Prospectively collecting tissue for a study is expensive taking many years, whereas Neuropathology archives in the UK National Health Service (NHS) contain a wealth of tissue collected over 40-50 years of diagnostic surgical and autopsy practice. ~ 500,000 archived cases exist, 18,500 added annually, including tumor, muscle and nerve biopsies and diverse CNS disorders. Objectives: To provide tissue for neuroscience research and reduce time for researchers to gain ethical approval. Methods: UK Neuropathologists collaborate in a national virtual brain bank involving 26 regional clinical neuroscience centers. BRAIN UK, supported by the British Neuropathological Society, funded by the Medical Research Council and Brain Tumor Research, is a matchmaker with generic ethical approval covering projects via a straightforward application process (www.brain-uk.org). A linked-anonymized database includes diagnosis and simple demographics. Results: Overall (2010 – 2020), 141 studies have been supported, more than 10,000 cases approved for release, resulting in 70 publications to date. Studies have encompassed a wide variety of conditions often not present in formal brain banks including head injury, epilepsy, psychiatric, genetic/developmental disorders, orphan diseases, neuroinflammation and neurodegenerative diseases. > 50 tumor studies have been supported, providing large numbers of cases or rare tumors, highlighting the success of this approach. Conclusion: BRAIN UK is a national virtual brain bank, facilitating access to under-utilized NHS neuropathology diagnostic archives for international researchers. Tissue that would otherwise have been unused has supported valuable neuroscience research.

Keywords: Neuropathology – tissue – brain bank

P216

Bacterial lipopolysaccharide (LPS) in cavernomas. An immunohistochemical study

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Introduction: Cerebral cavernomas are a cluster of unmatured dilated vascular structures that pose a risk for seizures, hemorrhage and neurological deficits. Three mutations causing cavernous malformations have been identified but they do not explain all cases. The clinical course varies with similar genetic backgrounds which suggests that there may be additional factors affecting the clinical course and pathogenesis such as immunological factors. Previous research has shown that bacterial lipopolysaccharide (LPS) is connected to the pathogenesis of cavernomas. Objectives: We investigated whether exposure to microbes modulates the inflammatory response present in the cavernoma wall through transmigration of bacteria-derived molecules to the lesion. We hypothesize that the presence of possibly GI tract derived LPS in the cavernomas affects possibly via TLR activation the clinical course and

pathogenesis. Methods: Tissue was obtained from surgery of 17 cavernomas and 3 postmortem Circulus Willisi. The tissue samples were studied with immunohistochemistry. Results: LPS was found in 10/17 (59%) of all cavernomas and was localized in endothelium in 9/17 and in smooth muscle layer 4/17 of the cavernomas. There were LPS-positive inflammatory cells in 6/17 of the cavernomas and 3/6 of those were located perivascularly. LPS-positivity was found in the Circulus Willisi endothelium and smooth muscle layer, but not perivascularly. Conclusion: We confirm that some cavernomas have LPS migrated to vascular wall and perivascular sites. LPS-positivity in CCM walls may suggest that LPS has a role in cavernoma pathogenesis possibly via TLR-activation. Further studies are needed for the evaluation of the pathobiological process and clinical significance.

Keywords: Cavernoma – lipopolysaccharide – vascular malformation

P217

Lipopolysaccharide is expressed in the inflammatory cells of brain AVMs and associates with COX2 expression

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Introduction: Brain AVMs (bAVMs) are vascular anomalies with a strong inflammatory component. Our previous study confirmed COX2-PGE2-NFkB signaling pathway in bAVMs, indicating inflammation derived vascular remodeling behind bAVMs' development. The inflammation's etiology is not understood but it has been suspected to make bAVMs more rupture prone. Recently, bacterial flora was found to be connected to development of vascular anomalies, possibly through inflammation. Objectives: The aim of this study was to verify, whether bacterial lipopolysaccharide (LPS) is expressed in bAVMs and whether it triggers LPS-TLR4-Myd88 signaling and influences bAVMs' inflammation and clinical course. Methods: Surgical samples of 81 bAVMs from Kuopio University Hospital were stained immunohistochemically. Circles of Willis (n = 5) were used as control. Clinical data was collected from patient records. Results: In circles of Willis, LPS, TLR4 and Myd88 were expressed in endothelial and smooth muscle cells. In bAVMs, LPS was found also in perivascular inflammatory cells in 63% (51/81) of the bAVMs and the overall LPS expression correlated with samples' COX2 expression ($r = 0.344$, $p = 0.014$, Spearman rank correlation). LPS expression did not associate with patients' rupture status. TLR4 was found focally in 88% (72/82) and Myd88 in 84% (66/79) of the bAVMs and these molecules were seen in all cell types. Conclusion: We confirm that systemic microbial parts are found in bAVM inflammatory cells and that this associates with expression of COX2, which is an important mediator of inflammation. This offers a potential explanation to the inflammation seen bAVMs. LPS seems to, at least partly, act through TLR4-Myd88 signaling.

Keywords: Brain arteriovenous malformation – lipopolysaccharide – cyclo-oxygenase 2 – intracranial hemorrhage

P218

Vanadium, an environmental pollutant, induces oxidative damage in the brain following acute exposure

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Introduction: Vanadium, an environmental pollutant, induces oxidative damage in the brain following acute exposure. Purpose: This study was designed to ascertain neurodegenerative consequences of chronic vanadium administration and withdrawal after initial exposure. Methods: A total of 85 four weeks male BALB/c mice were used and randomly divided into three major groups of vanadium treated (sodium metavanadate 3 mg/kg, intraperitoneally (i.p), 0 – 18 months), matched controls and animals that were exposed to vanadium for 3 months and thereafter withdrawn from exposure. Mice were thereafter sacrificed at different time points. Metal profiling was done using Laser Ablation Inductively Coupled Plasma Mass Spectrometry. Paraffin sections of the brain were stained with Hematoxylin and Eosin, and immuno-histochemically probed to demonstrate Microglia, Astrocytes, and neurons (including nuclear morphology and cytological outline). Results: Metal profiling revealed increasing vanadium absorption with regional variabilities following chronic exposure. but the brains had diminishing residues of the metal after withdrawal. There was a progressive disruption of layering pattern, degeneration, and necrosis in the prefrontal cortex, hippocampal pyramidal cells, and Purkinje cells of the cerebellum in vanadium-exposed brains. With prolonged exposure (15 – 18 months), the evident neuropathology was microgliosis, while progressive astrogliosis was observed till 12 months. All these cellular changes were ameliorated after vanadium withdrawal. Chronic administration of vanadium in mice resulted in brain damage and metal accumulation which showed regional variabilities with time. The metal profile and pathological effects

were not completely eliminated from the brain even after a long time of withdrawal from vanadium metal.

Keywords: Vanadium – chronic neurotoxicity – cognitive deficit – neuro-inflammation

P219

Application of Raman spectroscopy for detection of histologically distinct areas in formalin-fixed paraffin-embedded (FFPE) glioblastoma

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Background: Although microscopic assessment is still the diagnostic gold standard in pathology, non-light microscopic methods such as new imaging methods and molecular pathology have considerably contributed to more precise diagnostics. As an upcoming method, Raman spectroscopy (RS) offers a "molecular fingerprint" which could be used to differentiate tissue heterogeneity or diagnostic entities. RS has been successfully applied on fresh and frozen tissue, however more aggressively, chemically treated tissue such as formalin-fixed, paraffin-embedded (FFPE) samples are challenging for RS. Methods: To address this issue, we examined FFPE samples of morphologically highly heterogeneous glioblastoma (GBM) using RS in order to classify histologically defined GBM areas according to RS spectral properties. We have set up a SVM (support vector machine)-based classifier in a training cohort and corroborated our findings in a validation cohort. Results: Our trained classifier identified distinct histological areas such as tumor core and necrosis in GBM with an overall accuracy of 70.5% based on spectral properties of RS. With an absolute misclassification of 21 out of 471 Raman measurements, our classifier has the property of precisely distinguishing between normal appearing brain tissue and necrosis. When verifying the suitability of our classifier system in a second independent dataset we detected no overlap between necrosis and normal appearing brain tissue. Conclusion: These findings show that histologically highly variable samples such as GBM can be reliably recognized by their spectral properties using RS. As a conclusion, we propose RS as a potential future additional method in the pathological toolbox for tumor diagnostics.

Keywords: Raman spectroscopy – FFPE – glioblastoma – machine learning – pathology

P220

Convential diagnostic methods and ex vivo confocal laserendomicroscopy (CLE) in neuropathology: a comparison

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Introduction: Intraoperative information about a tumor entity is of the utmost importance in the surgical treatment of unknown mass. Confocal endomicroscopy combined with fluorescence agents offers the possibility of imaging of fresh tissues at cellular and subcellular resolution and therefore suitable for rapid clinical decision making. Objectives: In this investigation we aim to provide further insight into the ex vivo use of CLE for intraoperative identification of the various histological patterns of different tumor entities. Methods: During the surgical opening of the dura mater, 5 mg per kg body weight of sodium fluorescein were intravenously applied to 40 participating patients. Shortly after injecting the fluorescent agent, a sample of the suspicious lesion was given to a neuropathologist who first examined the fresh tissue ex vivo using CLE, second in H&E stained cryosection, third in methylene-blue smear preparation and last in formalin-fixed, paraffin embedded and H&E stained tissue. Results: It was possible to detect a similarity of patterns between an overall of 4000 pictures taken by CLE and the conventional microscopical examination of the same tissue. Further, we were able to identify pathologies in all cases and formulate a correct diagnosis in most cases of patients with neoplasia like gliomas, meningiomas, metastasis and radiation inflammation and avital cystic tissue. Conclusion: CLE is a potential new neuropathological tool for real-time diagnostics in the operating theatre. The advantage of rapid examination in situ and thus the presentation of conspicuous tissue in its living environment needs to be investigated further.

Keywords: Confocal laser endomicroscopy – brain tumors – real-time intraoperative diagnosis

P221

Postmortem neuropathologic examination of a 5-case series of CAR T cell treated patients

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Introduction: Chimeric antigen receptor (CAR) T cell therapy is a promising immunotherapy for the treatment of refractory hematopoietic malignancies. Among its adverse events, neurotoxicity is one of the most important. Its exact physiopathology is unknown and neuropathologic information is scarce. Objectives: We aimed to describe the neuropathologic changes found in brains from patients that received CAR T cell therapy at our institution. Methods: Postmortem examination of five brains from patients that underwent CAR T cell therapy from 2017 to 2020. Histologic examination and Polymerase Chain Reaction (PCR) in paraffin blocks for the detection of CAR T cells were performed. Results: Two patients died of relapse of their base malignancies, and the rest died of infection, cytokine release syndrome and encephalopathy of unknown etiology. The last case showed a severe perivascular and interstitial lymphocytic infiltration, predominantly CD8+, admixed with a diffuse interstitial histiocytic infiltration, affecting mainly the spinal cord, midbrain and hippocampus, along with a diffuse gliosis of basal ganglia, hippocampus and brainstem. Microbiologic studies were negative for neurotropic viruses, and PCR failed to detect CAR T cells. The rest of the cases showed a mild patchy gliosis and microglial activation, with no other remarkable histologic changes. CAR T cells were detected by PCR in only one case. Conclusion: Patients that die after CAR T cell therapy show unspecific or minimal neuropathological changes. CAR T cell related toxicity may not be the only cause for neurological symptoms, and autopsy may detect other pathological processes.

Keywords: CAR T cell – neurotoxicity – cytokine release syndrome

P222

Histopathological changes and CSF findings after natalizumab therapy for multiple sclerosis

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Background: Natalizumab is a highly beneficial drug for the treatment of relapsing-remitting multiple sclerosis (MS). However, some patients do not respond to it, and some are at higher risk of developing progressive multifocal leukoencephalopathy (PML). Objectives: We aimed to characterize the inflammation in brain specimens and CSF after natalizumab therapy. Methods: We performed a detailed histological analysis of the central nervous system (CNS) inflammatory infiltrate of 24 brain specimens from natalizumab-treated patients, consisting of 20 biopsies and 4 autopsies and compared our results to 21 MS controls not treated with natalizumab. In addition, immune cells in blood and cerebrospinal fluid (CSF) of 30 natalizumab-treated patients were quantified by flow cytometry. Results: Inflammatory infiltrates within lesions were mainly composed of T cells and macrophages, some dendritic cells, B cells and plasma cells. There was no significant difference in the numbers of T cells, macrophages and microglia cells in lesions of natalizumab-treated patients as compared to controls. Dendritic cells were reduced with longer ongoing therapy duration. Plasma cells were significantly increased in active demyelinating lesions, but no correlation to clinical disability was observed. Conclusion: Natalizumab does not completely prevent immune cells from entering the CNS and even leads to an accumulation of plasma cells, the clinical significance of which is not known. As B cells are considered to serve as a reservoir of the JC virus, the observed plasma cell accumulation and reduction in dendritic cells in the CNS of natalizumab-treated patients may play a role in PML development.

Keywords: Multiple sclerosis – natalizumab – tysabri – plasma cells – progressive multifocal leukoencephalopathy

P223

Human post-mortem organotypic brain slices to study leukodystrophies

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Background: Leukodystrophies are inherited disorders characterized by the predominant involvement of the central nervous system white matter. Due to the complex mechanisms driving the pathogenesis of these disorders, there is an urgent need to develop models in which one can assess the molecular and cellular interplay between multiple cell types. Objective: We aimed at developing an ex vivo organotypic brain slice method using post-mortem human brain tissue to study leukodystrophy disease mechanisms. We evaluated whether the cultured slices recapitulate the known neuropathological characteristics and how long they survive ex vivo. Additionally, we sought to de-

termine whether culturing in human cerebrospinal fluid (CSF) increases tissue viability. Methods: Post-mortem brain tissue was obtained at autopsy from five leukodystrophy patients and six unrelated control subjects. Organotypic slices of 300 µm-thick were cut using a vibratome and cultured onto semi-porous membrane inserts up to six weeks. Human CSF was collected post-mortem and added to the culture medium in a 50% concentration. (Immuno-)Histological analyses and a cytotoxicity assay were employed to assess disease-specific neuropathological characteristics and determine tissue viability. Results: Human organotypic slices survive up to six weeks in vitro and tissue structure remains well preserved. Preliminary data indicate that addition of human CSF improves tissue viability. Organotypic slices of an adult vanishing white matter patient show dysmorphic astrocytes, lack of myelin, and relative preservation of axons, corresponding to what is found in adult patients. Conclusion: Human organotypic slices survive for several weeks in vitro and may represent a model suitable for studying leukodystrophy disease mechanisms.

Keywords: Leukodystrophy – organotypic brain slice cultures – post-mortem

P224

Single injection of gadolinium based contrast agents does not induce significant alterations of gene expression in rodent cerebellum

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Background: The finding of gadolinium (Gd) deposition in the brain, first and foremost in the dentate nucleus in the cerebellum, following contrast enhanced MRI, raised awareness of potential adverse effects of (GBCA) administration. Due to previous findings in in vitro experiments, a conceivable side-effect of Gd deposition could be an altered gene expression. Thus, we aimed to investigate the influence of GBCA administration on gene expression in the cerebellum of mice. Methods: Three groups of eight mice were intravenously injected with either linear gadodiamide, macrocyclic gadoterate or saline (NaCl 0.9%) in a 10× clinical dosage (1 mmol GBCA/kg body weight). Animals were euthanized four weeks after injection and whole genome gene expression analysis and Gd quantification of the cerebellum were performed. Results: After administration of a single dose of GBCAs, traces of linear and macrocyclic GBCAs were detectable in the cerebellum. Unsupervised sample clustering using principal component analysis (PCA) did not reveal treatment related clustering. Gene expression analysis revealed six significantly differentially expressed genes, of which only two are functionally annotated: Crym and Prss22. Conclusion: Four weeks after single application of GBCAs no major alterations of gene expression in the cerebellum are seen. Thus, application of GBCAs, especially linear GBCAs may lead to depositions in the brain but do not lead to specific disturbances on RNA level in mice.

Keywords: Gadolinium deposition – gene expression analysis – GBCA

P225

Fluorescence labeled PARP inhibitor PARPi-FL for intraoperative identification of tumor cells in human glioblastomas - a pilot study

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Introduction: Glioblastoma surgery is associated with high rates of positive margins. Imaging could help identify tumor cells, although currently available imaging approaches lack molecular specificity. Fluorescent Poly-(ADP-Ribose)-Polymerase (PARP) inhibitor could be a good candidate since PARP1 plays an important role in DNA-repair and is highly overexpressed in many tumors, including glioblastomas. Objectives: We performed a pilot study to investigate the specificity of a fluorescence labeled PARP-inhibitor (PARPi-FL) for tumor cells from human glioblastomas with a particular interest in its use for intraoperative diagnostics in fluorescence guided confocal laser endomicroscopy (CLE). Methods: We investigated a series of brain tumors (n = 13) that were resected without fluorescence guidance. Fresh tumor samples were topically stained ex vivo with PARPi-FL for 3 – 5 minutes and imaged using a CLE Convivo© (Carl Zeiss AG, Germany). Subsequently, cryostat sections were performed for conventional H&E intraoperative diagnostics. All results were verified by FFPE histopathology. Results: We identified a strong and specific PARPi-FL staining in glioblastomas and metastases, whereas meningiomas and reactive lesions showed no enrichment and were therefore PARPi-FL negative. Moreover, especially at the resection margins of glioblastomas, different cell types, most likely tumor cells and cells of the microenvironment, could be identified on the basis of PARPi-FL positivity. Conclusion: There is a genuine demand for tumor cell markers for intraoperative decision making especially at resection margins. In this proof-of-principle study we showed that PARPi-FL is a promising imaging agent for intraoperative

identification of positive margins in glioblastoma and other malignant brain tumors.

Keywords: PARPi – CLE – confocal endomicroscopy – in vivo imaging – intraoperative diagnostics – neurosurgery

P226

Digital pathology in the muscle biopsy services during the COVID-19 pandemic: a large UK center experience

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Introduction: In March 2020, the WHO declared the COVID-19 outbreak as a pandemic and soon after the UK governments declared "lockdown" measures. In line with the UK General Medical Council guidance to the healthcare workers, we adopted new working practices to meet the need for flexible and remote working including virtual clinical meetings. Methods and adaptations: The "fresh" muscle biopsy sample was handled with necessary precautions as per the Public Health England safe handling guidance. The whole slide digital images were acquired using an advanced digital slide scanner. The images were analyzed on a virtual microscopy platform with high resolution PC monitor. The digital pathology reporting was introduced as per the UK Royal College of Pathologists guidelines. Initial self-validation, comparing digital images with the respective glass slides was undertaken. To maintain the clinical standards and patient safety, whenever in doubt, we concurred with the glass slide findings. The multi-disciplinary neuromuscular team meetings and clinical discussions were held on a virtual platform by taking appropriate steps to protect patient confidentiality. Results: The digital reporting has enabled quicker turn around. The virtual meeting platform has enabled expansion of clinical audience and participation. It has facilitated better training opportunities to the junior doctors. Despite intermittent challenges such as scan failure, poor quality audio/video, the overall end-user feedback has been very positive. Conclusion: The COVID-19 has brought about several changes. We share our novel experience over the last year, which we hope will help to improve diagnosis and treatment in the neuromuscular services.

Keywords: Muscle biopsy digital pathology

P227

The neurovascular unit in leukodystrophies: new insights from neuropathology

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The blood-brain barrier is a dynamic barrier in the microvasculature of the central nervous system that separates the blood from the brain parenchyma. Its main roles are to maintain brain homeostasis, protect the brain from harmful compounds and cells, and actively regulate nutrient influx and waste efflux. The blood-brain barrier is composed of specialized brain endothelial cells sealed by tight junctions. Together with these, astrocytes, neurons and pericytes compose the neurovascular unit and interact to maintain blood-brain barrier function. Dysfunction of one of these cell types can contribute to neurodegeneration. Leukodystrophies are genetic white matter disorders characterized by predominant involvement of the brain white matter. Their clinical course is mostly progressive and often there is no cure. Many leukodystrophies exhibit neuropathological features and/or involvement of disrupted signaling pathways that could contribute to dysfunction of the neurovascular unit, including reactive gliosis, neuroinflammation and vascular calcifications. In contrast to the building knowledge supporting a role for neurovascular unit dysfunction in grey matter disorders, little if any research has been conducted on this subject in leukodystrophies. The aim of this review is to provide further insights into the pathology of the neurovascular unit in leukodystrophies. This knowledge can pave the way to better understanding the disease mechanism underlying leukodystrophies.

Keywords: Leukodystrophy – neurovascular unit – white matter – astrocytes – endothelium – pericyte – microglia

P228

Plasticity of cholinergic system in the spinal cord following traumatic brain injury

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Introduction: Mechanism of motor deficit due to traumatic brain injury (TBI) is poorly understood. Previ-

ous animal experiments have shown unilateral injuries to the sensorimotor cortex produce postural asymmetry, but the underlying mechanism is unclear. The cholinergic change appearing in the spinal cord motor neurons (MNs) may play a role in this mechanism. Methods: In this study we examined what effect a TBI on the right side hindlimb sensorimotor cortex may have on cholinergic expression on MNs after 3, 14, and 28 days and corresponding sham rats (controls). Hindlimb postural asymmetry of TBI and sham was measured before perfusion. The lumbar spinal cord was removed and sectioned. One set of sections were immunolabeled with anti-choline acetyltransferase (ChAT) antibody. The optical density of ChAT labeled-MNs was analyzed. To investigate possible changes of number of cholinergic boutons on MNs in reaction to the TBI. Another set of sections were labeled with anti-vesicular acetylcholine transporter (VAChT) antibodies. Results: We found that right-side TBI generated left hindlimb postural asymmetry. The density of cholinergic immunoreactivity on MNs showed no significant difference between ipsilateral and contralateral side in TBI and sham in all groups. VAChT labeling after 3-days showed that the number of VAChT-positive boutons on the MNs was changed between the ipsilateral and contralateral side in TBI rats. Conclusion: The results indicate that the flexion of the hindlimb caused by TBI is not due to the increased excitability of MNs per se. It may partly be due to the increased synaptic input to the MNs from local cholinergic interneurons.

Keywords: Motor neurons TBI Spinal cord

P229

Diagnosis of FXTAS in the biopsy of a patient under study for leukoencephalopathy

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Introduction: Fragile-X Tremor-Ataxia Syndrome (FXTAS) is caused by an expansion of the CGG trinucleotide repeats in the non-coding region of the Fragile X Mental Retardation 1 (FMR1) gene on the X chromosome. Patients with more than 200 repeats develop a fragile X syndrome, while patients with 55 – 200 repeats may present neurodevelopmental disorders and FXTAS, the latter more common in men from the seventh decade of life and with characteristic neuropathological findings. Objectives: We present the case of a man with ataxia with altered white matter who underwent a brain biopsy with neuropathological findings of FXTAS. Methods: A 79-year-old man with a history of bladder carcinoma treated with chemotherapy who presented ataxia of one year of progression. Magnetic resonance imaging showed extensive involvement of the frontal white matter, the cerebellum and the brain stem. A brain biopsy of the right frontal region was performed to study leukoencephalopathy. Results: The biopsy showed an intense vacuolization of the white matter with loss of myelin together with frequent intranuclear eosinophilic inclusions mainly in astrocytes, some of them enlarged, which were positive with p62 and ubiquitin. A genetic study was performed in the peripheral blood that showed 110 CGG repeats in the FMR1 gene. Conclusion: Although the diagnosis of FXTAS is mainly clinical and genetic, we must include it in the differential diagnosis of brain biopsies for the study of leukoencephalopathy given its characteristic neuropathological findings.

Keywords: Leukoencephalopathy – FXTAS – p62

P230

Astrocyte-oligodendrocyte-microglia crosstalk in astrocytopathies

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Introduction: Defective astrocyte function due to a genetic mutation can have major consequences for microglia and oligodendrocyte physiology, which in turn affects the white matter integrity of the brain. Purpose and results: This review addresses the current knowledge on shared and unique pathophysiological mechanisms of astrocytopathies, including vanishing white matter, Alexander disease, megalencephalic leukoencephalopathy with subcortical cysts, Aicardi-Goutières syndrome, and oculodentodigital dysplasia. The mechanisms of disease include protein accumulation, unbalanced secretion of extracellular matrix proteins, pro- and anti-inflammatory molecules, cytokines and chemokines by astrocytes, as well as an altered gap junctional network and a changed ionic and nutrient homeostasis. Interestingly, the extent to which astrogliosis and microgliosis are present in these astrocytopathies is highly variable. Conclusion: An improved understanding of astrocyte–microglia–oligodendrocyte crosstalk might ultimately lead to the identification of druggable targets for these, currently untreatable, severe conditions.

Keywords: Astrocytopathies – cellular crosstalk – astrocytes – oligodendrocytes – microglia

P230Z

Altered matrilin-2 and tenascin-C expression in corneal diseases with reduced transparency

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Purpose: The purpose of this study is to examine the expression of tenascin-C and matrilin-2 in three different disorders, which frequently require corneal transplantation. These pathological conditions include bullous keratopathy (BK), Fuchs' endothelial corneal dystrophy (FECD) and corneal scaring in herpetic keratitis. Materials and methods: Histological sections of corneal buttons removed during keratoplasty were analyzed in BK (n = 20), FECD (n = 9), herpetic keratitis (n = 12), and cadaveric control (n = 10) groups by light microscopy following chromogenic immunohistochemistry. The sections were evaluated by three investigators and semiquantitative scoring (0 to 3+) was applied according to standardized methods at 400× magnification. Each layer of the cornea was investigated, moreover, the stroma was subdivided into subepithelial, middle, and the pre-Descemet areas for more detailed analysis. Results: Excessive epithelial and stromal expression of tenascin-C was identified in all investigated conditions, the results being most pronounced in the pre-Descemet layer. Regarding matrilin-2, when examined in BK, there was increased labelling intensity in the epithelium ($p < 0.001$) and stromal layers ($p < 0.05$), and a decrease in the endothelium ($p < 0.001$). In the other investigated conditions only a low degree of stromal localization ($p < 0.05$) of matrilin-2 was detected. Conclusion: The expression of tenascin-C and matrilin-2 differs when examined in various corneal pathologies resulting in opacification. Both molecules seem to be involved in the regeneration and wound healing of the corneal matrix in these diseases.

Keywords: Corneal extracellular matrix – corneal opacity – immunohistochemistry – matrilin-2 – tenascin-Cf

COVID-19

P231/SY 13.4

Post-infectious myopathies related to COVID-19

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Introduction: SARS-COV2 infection has come to focus in 2020, when COVID-19 was declared pandemic worldwide. In addition to interstitial pneumonia, its physiopathology involves multiple mechanisms, mainly inflammatory dysregulation and thrombo-inflammation, affecting most tissues and organs. Unexpected manifestations have been described, both in the acute phase and later, as a post-infectious disease. Case descriptions: Our study focuses on muscle biopsies obtained from 4 post-COVID-19 patients who had recovered from the infection, when muscle symptoms emerged, all with very high CPK levels. Two were females, 7th decade of life, presenting mild to moderate respiratory symptoms that resolved within a few days. About a month later they developed weakness and biopsies showed necrotic fibers in different proportions, one with features of rhabdomyolysis. Treatment with immunosuppressants and immunoglobulin resulted in significant improvement in motor and sensory conditions. The third patient, a 49-year-old hypertensive, obese and diabetic male, had severe respiratory symptoms requiring orotracheal intubation. Discharged without symptoms, he developed severe muscle weakness and tetraparesis one month after the COVID-19 onset. Muscle biopsy showed degenerate and regenerating fibers and CD8 lymphocytic infiltration. Immunostaining for SARS-COV2 was negative. Treatment with methylprednisolone and immunoglobulin was followed by improvement of symptoms, of inflammatory markers, and decrease in muscle enzymes. The forth one, a 53-year-old hypertensive and diabetic male, developed weakness and skin lesions 3 – 4 weeks after COVID-19 diagnosis. Muscle biopsy showed perifascicular atrophy and myofiber vacuolization, suggestive of dermatomyositis. Conclusion: Our observations, in correlation with clinical history, biopsy findings and negative immunohistochemical results for COVID-19, strongly suggest the possibility of post-infectious myopathies.

Keywords: COVID-19 – myopathies – myositis – biopsy

P232

Spectrum of neuropathological findings in a series of 13 COVID-19 patients

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Introduction: COVID-19 has been associated with a broad spectrum of abnormalities in the central nervous system including inflammatory changes and coagulopathy. Methods: We present the neuropathological findings in the brains of 13 COVID-19 patients. We included the clinical history with focus on immunological status. Our neuropathological workup included sampling of representative brain areas with olfactory bulb, meninges and choroid plexus. Histological and immunohistological stainings for inflammation including activated microglia, for cerebrovascular pathology, as well as an immunohistochemical panel for neurodegenerative disorders were performed. Mean age of our cohort was 68 [33; 90]. 4 patients had a previous diagnosis of a neurodegenerative disorder. Results: Postmortem evaluation revealed severe meningoencephalitis with multiple hemorrhagic infarctions (n = 1), multiple hemorrhagic infarctions (n = 1), major hemorrhages (n = 2) and hypoxic encephalopathy (n = 1). Isolated bulbar and meningeal inflammation was found in 3 patients. 5 patients did not have any neuropathological changes. Our neurodegenerative analysis detected FTLD-TDP, Alzheimer's disease neuropathological changes and hippocampal sclerosis. Conclusion: Our cohort confirms the variety of neuropathological findings in patients with COVID-19 with a range from isolated bulbitis/meningitis to full blown meningo-encephalitis with hemorrhagic infarcts. The pleiad in symptomatology suggests that other factors should be taken into account, considering the vulnerability of cerebral involvement as to the immunological status and the coagulopathy.

Keywords: COVID-19 – neuropathology – inflammation – coagulopathy

P233

SARS-CoV-2 neuropathology: evidence from a post-mortem autopsy series in Padova, Italy

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Introduction: SARS-CoV-2 is a novel strain of Coronavirus that mainly targets the respiratory tract, but with important implications also for the CNS. Data deriving from autopsy studies supports the neuroinvasive potential of SARS-CoV-2, even though infection appears to be limited to sparse cells within the brainstem and was not associated with the severity of neuropathological changes. Objectives: In the following study, we assess the neuropathological changes of 14 patients who died following a diagnosis of Sars-CoV-2 infection in Padova, Italy from March 2020 to January 2021. Methods: The cerebrum, cerebellum, brainstem, cranial nerves and meninges were sampled and histopathological evaluation was performed by histochemistry and immunohistochemistry for GFAP, CD8, CD61, CD68 and HLA-DR antibodies. SARS-CoV-2 proteins and RNA were investigated through immunohistochemistry, RT-PCR and in-situ hybridization. Results: Small vessel thromboses were identified in two patients, while fresh territory ischaemic lesions were identified in three patients. Astrogliosis and microglial activation were more pronounced at the level of the brainstem in all subjects. SARS-CoV-2 proteins were found within the brainstem and meninges of 4 patients. In one patient, SARS-CoV-2 proteins and RNA were identified throughout the whole rostrocaudal extent of the brainstem and basal ganglia, with prominent involvement of neurons and oligodendrocytes in the mesencephalon, rostral pons and medulla. Conclusion: Although limited by the number of our cohort, the study contributes to define the neuroinvasive potential of SARS-CoV-2 within the CNS. In line with available literature, SARS-CoV-2 invasion does not appear to correlate with the severity of neuropathological changes.

Keywords: COVID-19 – neuropathology – neurotropism – SARS-CoV-2

P234/SY 13.3

Olfactory transmucosal SARS-CoV-2 invasion as port of central nervous system entry in COVID-19

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Introduction: Coronavirus disease 2019 (COVID-19) is a pandemic respiratory disease which is accompanied by a broad spectrum of neurological manifestations in more than one-third of COVID-19 cases. For the latter, olfactory and gustatory disturbances such as anosmia and ageusia, are often leading symptoms of SARS-CoV-2 infection. Given the close proximity of neuronal and supporting cells the olfactory mucosa as a neural-mucosal interface may represent a potential port of CNS entry for SARS-CoV-2. Objectives: Identifying potential sites of SARS-COV-2 CNS entry and morphological changes associated with SARS-CoV-2 infection. Methods: We systematically investigated postmortem tissue of the CNS and nasopharynx from 75 individuals with COVID-19. Based on the correlation of clinical data and (neuro-) pathological examinations, SARS-CoV-2-specific morphological changes were determined. Using quantitative real-time PCR, RNAscope in situ hybridization, immunohistochemistry and electron microscopy, we characterized the CNS tropism of SARS-CoV-2 and the consequences thereof. Results: Acute thromboembolic ischemic infarcts (n = 10/64) and a strong innate immune response, mediated by HLA-DR+ microglia with a linked increase in proinflammatory mediators in the cerebrospinal fluid, are leading alterations in the CNS. Besides, a distinct immunoreactivity for SARS-CoV Spike protein was found in the olfactory epithelium - here co-localizing with neural/neuronal cells - and cerebral endothelial cells. We were also able to illustrate intact coronavirus particles in the olfactory mucosa ultrastructurally. Conclusion: SARS-CoV-2 can enter the nervous system by crossing the neural-mucosal interface in the olfactory mucosa. SARS-CoV-2 infection results in an innate immune response with activation of HLA-DR+ microglia and increased levels of inflammatory mediators in the CNS.

Keywords: SARS-CoV-2 – COVID-19 – CNS – olfactory mucosa – innate immune response

Late Abstracts

P235

Histological characteristics and proliferative activity of glioblastomas in the central and peripheral regions of the tumor

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Introduction: Glioblastomas are the most common tumors of the central nervous system in adults. Pronounced heterogeneity in glioblastomas is revealed during histological examination. Objective: Compare histological structure and Ki-67 in the central and marginal areas of glioblastoma. Methods. Surgical material from 9 patients with glioblastomas (43 fragments from the center and the edge of the tumor). It was stained with H&E. IHC markers were used: GFAP, Ki-67. The Ki-67 level was calculated by a semi-automatic method using the ImageJ. Results. In 17 fragments macroscopically assigned to the center revealed a glioblastoma. Of the 26 slices regarded macroscopically by the marginal and perifocal zones, histological picture of anaplastic astrocytoma was revealed in 3 glasses, diffuse astrocytoma in 2, the perifocal zone in 2 and in 19 - a combination of plots with picture of glioblastoma and foci of perifocal zone. The average ki-67 value in 3 cases in the central areas was higher. In 2 patients, the average ki-67 level in the center was lower. The maximum Ki-67 level was higher in the center at 7 of 9 cases. In one case there were no differences. In another, the maximum Ki-67 value was higher in the edge fragments. Conclusion: The most informative sites are fragments from the central part of glioblastoma. In fragments from the edge and perifocal zone of the tumor, sites with varying degrees of anaplasia from intact brain, diffuse astrocytoma to anaplastic astrocytoma and glioblastoma can be observed, which is explained by the infiltrative nature of the growth of glial tumors.

Keywords: glioblastoma, ki-67 – intratumoral heterogeneity

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Piloid-like changes after combination therapy in glioblastoma with prominent gemistocytic component: a case report

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Introduction: Glioblastoma, a high-grade, diffuse astrocytic tumor variant. Gemistocytic astrocytes can be detected in some glioblastomas especially around necrosis. Objectives:

Describe the rare type of reactive changes in gliomas after combination therapy. Methods: Histological study included hematoxylin and eosin staining. GFAP, Vimenti, NSE, NF, MGMT, EGFR, p53 and ki-67 were used for immunohistochemical staining. Results: A 66-year-old man was hospitalized in the neurosurgical department. MRI revealed a tumor of the left frontal lobe of the brain with perifocal swelling. Microsurgical total removal of a tumor performed with electrophysiological monitoring. Histological examination revealed glioblastoma with a prominent gemistocytic component, pseudopalisading necrosis and endothelial proliferation. The patient underwent radiation therapy and chemotherapy with temozolomide. A local tumor recurrence was detected 2 months after surgery. The tumor was removed again 3 months after the first operation. Histological examination revealed a picture of an astrocytic tumor of a diffuse structure with prominent reactive changes from small piloid-like cells. The tumor also revealed a few gemistocytes. Axons of tumor cells are underlined. Pronounced Rosenthal dystrophy and pseudopalisading necrosis of reduced cellular reactivity are noted in the tumor. Angiomatosis with pronounced hyalinosis of the walls of the vessels and proliferation of the endothelium with thrombosis of some vessels was revealed. The tumor revealed a positive staining with antibodies to GFAP, Vim, negative – NSE, NF, MGMT, EGFR, p53. The ki-67 was very low (1 – 2%). Glioblastoma with severe reactive changes was diagnosed. Conclusion: Piloid-like changes in glioblastoma are a rare type of reactive changes after combination therapy.

Keywords: Glioblastoma – reactive changes – chemotherapy

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Heterogeneity in pediatric hemispheric high-grade glioma: genetic landscape at primary and recurrence tumor

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Introduction: Hemispheric pediatric high-grade glioma (pHGG) comprise a heterogeneous group of tumors, comprising tumors with recurrent somatic mutations in H3F3A or H3.1/H3.2, SETD2 and infrequently IDH1/2 mutations. Another subset of pHGGs H3/IDH-wildtype has been recently divided into three molecular entities: RTK I, RTKII, and MYCN-amplified. Methods: We performed WES on matched primary and recurrent from 4 patients (range 0,6 – 9 years) with hemispheric high-grade glioma H3/IDH-wildtype. Results: Genetic analysis shows the presence of variants shared between primary and recurrent tumor and variants exclusive of one or the other. NSD1 variants were present at high frequency in our series (100%) and shared between the samples independently at primary or recurrence. NSD1 variants (c.5924T>A; p.Leu1975His and c.5993T>A; p.Met1998Lys) occurs within the highly conserved SET domain. All these variants are novel and not reported in any database. For all of them the in silico prediction tools estimated a high probability to be deleterious for the protein function. The novel variant NSD1 (c.5924T>A; p.Leu1975His) was present in 1/4 case at recurrence, and in 2/4 cases only at primary tumor. The novel variant NSD1 (c.5993T>A; p.Met1998Lys) in 1/4 case was present at primary and recurrent tumor and in 1/4 only at primary tumor. NSD1 is a histone lysine methyltransferase with functions of mono-and dimethyltransferase in H3K36. Conclusion: The presence of NSD1 variants only at recurrence suggest they can be subclonal, the presence in both primary and recurrence suggest they are early events and stable, and could be affected by treatment if present at primary and not recurrent tumor.

Keywords: Pediatric high-grade glioma – high-grade glioma H3/IDH-wildtype – NSD1gene

Company Abstracts

NanoString Abstracts

Regional and sub-regional neuroinflammatory differences in the brain of sporadic Creutzfeldt-Jakob disease patients

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We investigated the expression of 800 neuroinflammation-associated genes in frontal cortex and cerebellum of patients who suffered from different subtypes of sporadic Creutzfeldt-Jakob disease (sCJD) using NanoString nCounter technology, which allowed direct labelling and counting of target sequences and avoiding DNA amplification introduced bias. Take home message the data indicate: 1) brain region exclusive and sCJD-specific genes functionally involved in cells' differentiation, migration and proliferation, and 2) sub-regional differences in the strength of neuroinflammation. Thus, directing future studies towards cell subtypes characterization based on

their molecular phenotypes, functions and location.

Understanding neuropathological disorders using GeoMx® digital spatial profiler

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NanoString is a global leader in spatial gene expression. With the launch of the GeoMx® Digital Spatial Profiler (DSP), researchers now have the ability to view morphology of their tissue and define exactly where they want to detect changes in gene expression for whole transcriptome RNA or up to 100+ proteins. The GeoMx® platform comfortably works with both FF or FFPE tissue in both human and mouse. The recently launched Whole Transcriptome Atlas (WTA) provides full coverage spatial profiling of any target in any tissue. This high-plex spatial analysis of RNA technique has been selected Nature's Method of the Year for 2020 and allows neuropathologists to decipher the intricacy of neuropathological diseases by full transcriptome in situ analysis. This talk will highlight the technology behind the GeoMx® with a focus on various test cases to visualize high impact research applications.