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Abstracts

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PLATFORM 1: Neurodegenerative: Alzheimer

1

Alzheimer's and Primary Age-Related Tauopathy: Insights on Amyloid-Dependent & Independent Mechanisms of Tau Seeding and Tangle Formation

D Smirnov[1], D Browne[2], D Coughlin[1], Y Kim[1], A Unapanta[1], A Kraus[2], A Hiniker[1]
[1]UC San Diego; [2]Case Western Reserve University

Background: Primary Age-Related Tauopathy (PART) is characterized by Alzheimer's-type tau neurofibrillary tangle (NFT) pathology without significant beta-amyloid deposits, challenging the amyloid cascade hypothesis of Alzheimer's pathogenesis. Unlike Alzheimer's disease neuropathologic change (ADNC), which progresses to affect the neocortex in later stages, PART remains confined to the medial temporal lobe, strongly resembling the structure and distribution of NFT pathology in early-stage ADNC (Braak I-IV).
Methods: We used quantitative digital histology, 3R/4R tau real-time quaking-induced conversion (RT-QuIC) seeding assays, and immunoblotting to compare tau histopathologic density, tau seeding activity, and tau post-translational modifications (PTMs) between Braak-matched PART ($n = 17$, Thal phase 0-2) and ADNC ($n = 21$, Thal 3-5) in the hippocampus (HP) and midfrontal cortex (MF).
Results: Digital histology showed similar tau density in HP and MF ($p = 0.40$ and 0.42). However, while seeding in the HP was comparable between the groups ($p = 0.69$), MF seeding was higher in ADNC than PART ($\beta \pm SE = 1.31 \pm 0.37$ log(SD50), $p = 0.001$), providing moderate differentiation (AUC=0.76). Tau seeding in both HP and MF predicted cognitive performance and rate of decline on the Mini-Mental State Examination ($p < 0.05$). Region specific effects were observed with HP seeding associated exclusively with memory performance ($\beta \pm SE$-0.54±0.20, $p = 0.01$) and decline ($\beta \pm SE = -0.044 \pm 0.02$/year, $p = 0.048$), and MF seeding associated exclusively with executive function performance ($\beta \pm SE = -0.41 \pm 0.11$, $p = 0.001$) and decline ($\beta \pm SE = -0.046 \pm 0.017$/year, $p = 0.01$). MF Tau seeding was associated with amyloid staging ($p = 0.001$) and amyloid pathologic density ($p < 0.001$). Immunoblot analysis indicates distinct PTMs may distinguish seed-competent AD versus PART tau forms.
Conclusions: These findings suggest two mechanisms for the formation of tau NFTs: an amyloid-independent age-related mechanism in limbic structures, present in both PART and AD, and an amyloid-dependent mechanism primarily seen in the neocortex of ADNC patients. This further shows that tau seeding precedes overt MF pathology in ADNC, and highlights its value predicting early cognitive changes and differentiating PART from AD before observable cortical tau NFT pathology.

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Treatment interval dependent effects of aducanumab therapy on Alzheimer's disease neuropathology

S Patel[1], B Lopresti[2], J Lingler[2], O Lopez[2], J Kofler[2]
[1]University of Pittsburgh Medical Center; [2]University of Pittsburgh

Background: Based on the amyloid hypothesis of Alzheimer's disease (AD), several anti-amyloid monoclonal antibodies have been tested in clinical trials as disease-modifying therapies. While they are efficacious in removing amyloid from the brain as assessed by fluid and neuroimaging biomarkers, their clinical benefits have been modest at best. We report autopsy findings of two aducanumab-treated cases to illustrate the possibly transient nature of aducanumab-related treatment effects.
Methods: The first case was a 56-year-old female who was diagnosed with early-onset AD at age 50. She received high-dose aducanumab for 3 years with no clinical side effects, and died about 2.5 years after the last infusion. The second case was an 84-year-old male with a several-year history of Mild Cognitive Impairment (MCI) but high amyloid burden on PET imaging, who received low-dose aducanumab for 2.75 years, followed by a 2-year gap and then monthly high-dose infusions until his death. Due to further mild cognitive decline, his diagnosis was changed to AD one year prior to death.
Results: Postmortem examination of case one revealed advanced AD neuropathologic change (A3,B3,C3) with

widespread diffuse and neuritic plaques, but also demonstrated unusual dot-like amyloid deposits in a few brain regions. For case two, there was clearance of amyloid from most neocortical regions with only a few scattered dot-like remnants with occasional microglial clusters and only very focal high neuritic plaque density. The final diagnosis for case two was intermediate-level AD neuropathologic change (A2,B2,C3), but application of standard scoring criteria was challenging in this case.

Conclusions: Findings from these two autopsy cases appear to support early biomarker data of amyloid rebound after discontinuation of anti-amyloid therapy. The second case also illustrates challenges with applying standard NIA-AA scoring criteria to cases after disease-modifying therapy and warrants discussion of post-treatment adaptations.

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SORL1 p.R953C mutation is a heritable risk factor for Alzheimer disease

D Child[1], E Fazeli[2], S Buck[3], S Jayadev[3], T Bird[3], J Young[3], O Andersen[2], C Keene[3], C Latimer[3]
[1]Department of Laboratory Medicine and Pathology, University of Washington; [2]Aarhus University; [3]University of Washington

Background: Most genetic contributions to individual Alzheimer disease (AD) risk are poorly understood. AD family and cohort analyses have recently implicated variations in the gene SORL1, which regulates endosomal recycling and has key roles in amyloid precursor protein (APP) processing. However, the resulting clinical severity varies based on the specific variant. Here, we describe a family with a novel SORL1 missense mutation, p.R953C (c.2785C>T), associated with both late and early onset AD in two generations, and we elucidate the mutation's functional consequence to SORL1 protein.

Methods: Autopsy evaluation was performed according to standard NIA-AA protocols. In vitro assays employed transient overexpression of either wild type (WT) or SORL1 p.R953C protein in HEK293 and N2a cells, followed by western blotting, flow cytometry, and colocalization immunocytochemistry.

Results: Four out of 5 generation II family members were diagnosed with clinical AD and had age of onset ranging from mid 50s to early 70s, and each had high AD neuropathologic change (ADNC) by NIA-AA criteria. The SORL1 p.R953C mutation was identified in each case, and no other genetic risk factors were found. Limbic-predominant age-related TDP-43 encephalopathy (LATE) was also uniformly present, and Lewy body pathology occurred in three of four cases. Coincidentally, the unrelated, SORL1-negative parent of all generation II members also had high ADNC at autopsy but lacked concomitant proteinopathies. In vitro testing showed the mutant SORL1 isoform was associated with loss of SORL1 post-translational modification and secretion. There was prominent endoplasmic reticulum retention of the mutant isoform, with reduced SORL1 localization to early endosomes and cell surface.

Conclusions: These observations suggest that the SORL1 p.R953C mutation prevents SORL1 maturation, promotes abnormal intracellular localization, and significantly increases risk of AD. The cellular data indicate that this variant may lead to a loss of function of SORL1 as an endosomal receptor, which may also contribute to concomitant proteinopathies.

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Spatial proteomic comparison of hippocampal subregions in Alzheimer disease (AD) and primary age-related tauopathy (PART)

J Walker[1], M Orr[2], T Orr[2], E Woodoff-Leith[1], T Christie[1], K Farrell[1], J Crary[1], C White[3], R Teixeira-Yokoda[1], M Vij[1], E Daoud[3], T Kautz[4], E Selmanovic[1], T Richardson[1]
[1]Icahn School of Medicine at Mt. Sinai; [2]Wake Forest University; [3]UT Southwestern; [4]UT Health San Antonio

Background: Although Alzheimer disease (AD) and primary age-related tauopathy (PART) both harbor 3R/4R-tau immunopositive Alzheimer-type neurofibrillary degeneration, they differ in the spatial development of neurofibrillary tangles (NFTs) in the hippocampus. PART cases display an early predilection for neurofibrillary degeneration in the CA2 subregion, whereas AD typically develops NFTs in the entorhinal cortex and CA1 subregion initially.

Methods: Using Nanostring's GeoMx™ Digital Spatial Profiling (DSP), we analyzed expression levels of 73 proteins in NFT-bearing neurons, non-NFT-bearing neurons and background microenvironments in the entorhinal cortex, CA1 and CA2 hippocampal subregions.

Results: We found higher levels of synaptic and neuronal markers in NFT-bearing neurons in PART, such as synaptophysin, MAP2, calbindin and NRGN, as well as higher levels of proteins involved in degradation pathways, such as CTSD, Park7 and FUS. PART NFTs also displayed higher levels of neprilysin in CA2 and higher IDE and ADAM10 in CA1, all proteins that reduce β-amyloid deposition. AD demonstrated higher levels of several p-tau epitopes in non-NFT-bearing neurons. In the microenvironment, NEFL, NRGN, CLEC7a, GPNMB and CTSD were higher in PART, while p-tau S396 and BACE1 were higher in AD. When comparing subregions within disease states, synaptophysin, Park7, and GPNMB were higher in the CA1 subregion as compared to CA2 in PART, and GBA was higher in CA2. In AD, CLEC7A, CTSD and IDE were elevated in CA2 compared to CA1. In both AD and PART, calbindin and tyrosine hydroxylase were higher in CA2.

Conclusions: These findings demonstrate higher levels of neuronal and synaptic markers in PART, suggesting better maintenance of neuronal and synaptic integrity as compared to AD, as well as higher levels of proteins important for degradation of detrimental proteins. In addition, higher levels of

BACE1 in AD and higher levels of CTSD, Neprilysin, ADAM10 and IDE in PART may explain the differences in β-amyloid deposition in these entities.

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Cellular protein transport alterations in clinical dementia syndromes with pathological TDP43

K Ajroud[1], C Zamudio[2], J Lilek[2], C Spenser[2], A Matos[2], R Shapiro[2], B Danner[2], E Bambakidis[2], J Walker[3], T Richardson[3], E Pladies[4], J Gong[4], L Pan[4], K Bieniek[1], S Seshadri[1], L White[5], M Flanagan[1]
[1]University of Texas Health Center at San Antonio, Biggs Institute, San Antonio, Texas; [2]Northwestern University; [3]Mount Sinai; [4]Nanostring; [5]Pacific Health Research and Education Institute, Honolulu, Hawaii

Background: Dementia is characterized by the progressive loss of intellectual functioning. Alzheimer's disease (AD), is the most common form of dementia followed by vascular dementia (VD). It has also been shown that pathologic trans-active response DNA-binding protein 43 (pTDP43) is associated with cognitive decline. Others have suggested altered cellular protein transport results in pathologic protein accumulation. We aimed to identify alterations in protein transport in the development of dementia for pathologic AD, VD, and pTDP43.
Methods: Nanostring's GeoMx™ Digital Spatial Profiling Human Neuro nCounter protein panel was applied to human autopsy brains ($n = 11$) from the Nun Study and Honolulu Asia Aging Study in 74 ROIs to compare: 1) matched Tau tangles (pTDP43+ vs. pTDP43-); 2) matched Aβ (pTDP43+ vs. pTDP43-) matched AD neuropathologic change High with differing clinical profiles (dementia vs. cognitively intact); 4) primary age related tauopathy (PART) with differing clinical profiles (dementia vs. cognitively intact); 5) hippocampal sclerosis and pTDP43 + (with vs. without chronic microinfarcts). Entorhinal, CA1, CA2, and striatum were assessed with cell-type specific markers NeuN (neurons), iba1 (microglia), and GFAP (astrocytes). Mann-Whitney with BH correction was used (p value < 0.05 considered significant)
Results: Cathepsin D (CTSD), a cargo protein involved in Tau degradation, was decreased in pTDP43+ groups in CA1 and dentate in neurons ($p = 0.03$), astrocytes ($p = 0.02$), and microglia ($p = 0.03$) in a background of pathologic AD. Striatal CTSD was decreased with co-existing chronic microinfarcts in a pTDP43+ background in microglia ($p = 0.01$) and astrocytes ($p = 0.01$). Hippocampal CTSD in neurons was decreased in PART with dementia when compared to cognitively intact PART ($p = 0.004$). In ADNC High cases, VPS35 (retromer complex) was decreased in those with dementia when compared to those who were cognitively intact ($p = 0.008$).
Conclusions: Results highlight regional CTSD, VPS35 and cell-type specific alterations in the setting of dementia, specifically in the presence of pTDP43.

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The neuropathological landscape of Hispanic and Non-Hispanic White Decedents with Alzheimer's disease

R Scalco[1], N Saito[1], L Beckett[1], M Nguyen[1], E Huie[1], H Wang[1], S Leskinen[2], D Flaherty[2], C DeCarli[1], R Rissman[3], A Teich[2], B Dugger[1]
[1]University of California Davis; [2]Columbia University; [3]University of California San Diego

Background: The United States' population is growing older and more diverse, leading to diversity among those affected by Alzheimer's Disease (AD). Most autopsy-based studies, however, have focused on cohorts of non-Hispanic White decedents (NHWD), with few studies including Hispanic decedents (HD).
Methods: We aimed to characterize the neuropathologic landscape of AD in NHWD ($n = 185$) and HD ($n = 92$) evaluated across three Alzheimer's Disease Research Centers (ADRCs): University of California San Diego, University of California Davis, and Columbia University. Only demented persons with a neuropathologic diagnosis of intermediate/high AD determined by NIA Reagan criteria were included. A frequency-balanced random sample without replacement was drawn from the NHWD group using a 2:1 age and sex matching scheme with HD. Four brain areas were evaluated: posterior hippocampus, frontal, temporal, and parietal cortices. Sections were stained with antibodies against Aβ (4G8) and phosphorylated tau (AT8). We compared the distribution and semi-quantitative densities for neurofibrillary tangles, neuropil threads, core, diffuse, and neuritic plaques. All evaluations were conducted by an expert blinded to demographics and group status.
Results: Wilcoxon's two-sample test revealed higher levels of neuritic plaques in the frontal cortex ($p = 0.02$) and neuropil threads ($p = 0.02$) in HD. Although the median was similar for both groups in the temporal cortex, the density of cored plaques was statistically greater in NHWD ($p = 0.02$). Results from ordinal logistic regression controlling for age, sex, and site of origin were similar. In other evaluated brain regions, semi-quantitative scores of plaques, tangles, and threads remained similar between groups with no statistical differences.
Conclusions: Our results demonstrate HD may be disproportionately burdened by AD-related pathologies in select anatomic regions, particularly tau deposits. Further research is warranted to understand the contributions of demographic, genetic, and environmental factors to heterogeneous pathological presentations.

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Post-Mortem Retinal Analyses of Amyloid-Beta Oligomers in Alzheimer's Disease

B Danner[1], C Zamudio[1], C Spencer[1], J Lilek[1], A Matos[1], M Morris[2], K Ajroud[3], M Flanagan[3]

[1]Northwestern University; [2]Johns Hopkins University; [3]UT Health San Antonio, Northwestern University

Background: Alzheimer's Disease (AD) is a progressive disorder characterized by memory impairment and abnormal behavior. Previous studies have shown that AβOs (Aβ oligomers) form clusters that eventually form amyloid aggregates in the brain. Aβ has also been shown to instigate several features of pathologic AD. The aim of this study is to quantify oligomeric Aβ in human retinal tissue, a region described to contain aggregated Aβ in AD progression. Aβ is a biomarker of (AD) in human models. While there are ongoing clinical trials of antibodies against oligomeric Aβ, our study is the first that quantifies AβOs in clinically and neuropathologically characterized postmortem retinal tissue.
Methods: Immunohistochemistry was performed on human retinal samples (n = 15) obtained from the Baltimore Longitudinal Study of Aging and Johns Hopkins University Alzheimer's Disease Center. These cases encompass the entire spectrum of AD as indicated by Thal, CERAD, and BRAAK stages. We used humanized, affinity-matured, IgG2 mAb selective biotinylated antibody (ACU-193) to detect soluble Aβ oligomers. Qupath quantitative pathology software was used to measure ACU193 immunoreactivity in digitized retinal whole slide images. Once percentage of positive tissue area was measured, one-way ANOVA and t-tests were performed to compare AβOs in controls and different stages of AD.
Results: When assessing ACU193 staining present from 14 retinal tissue samples, we observed 29-53% retinal tissue area positivity in controls and 15-72% retinal tissue area positivity in the AD group.
Conclusions: The retinal ACU193 immunohistochemical percent positivity observations in different stages of AD cases highlight the presence of ACU193 in the post-mortem human retinal in both healthy controls and in AD progression. The progression of well-established neuropathologically classified AD stages (e.g Braak) may correlate with retinal oligomeric Aβ levels. Larger sample numbers are necessary to comprehensively assess and quantify retinal oligomeric Aβ.

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Quantitative neuropathological comparisons between Down Syndrome and PSEN2

C Latimer, N Postupna, J Ariza-Torres, M Pacleb, M Damodarasamy, A Wilson, S Jayadev, B Thomas, C Keene
University of Washington

Background: The known genes responsible for familial Alzheimer's disease (FAD) are those involved in amyloid-β processing, including amyloid precursor protein (APP), presenilin 1 (PSEN1), and presenilin 2 (PSEN2). Down Syndrome (DS), which results in three copies of APP, is also highly associated with AD. While all types of FAD exhibit AD neuropathologic change, how these distinct genetic drivers of FAD differ in terms of the burden of amyloid (A) β and hyperphosphorylated tau pathology, and the mechanistic underpinnings, is unknown.
Methods: We previously reported the neuropathology of a 48-year-old decedent harboring both trisomy 21 (DS) and a PSEN2 N141I mutation. To better understand how this unique combination of FAD drivers influenced the neuropathology, we applied a battery of quantitative neuropathological assessments in this subject and matched brain donors with DS (n = 7) or PSEN2 variants (n = 6) alone. Quantitative measures include digital image analysis of pathologic proteins, gliosis, and neuroinflammation, extraction-based assays for amyloid and tau, and NanoString digital spatial proteomic profiling of amyloid plaques and microglia.
Results: Group comparisons revealed significantly higher amyloid burden and IBA1 immunoreactivity in DS compared to PSEN2 by image analysis, with a trend toward higher Aβ1-40 in tissue extracts. The combination of DS and a PSEN2 mutation results in a marked increase in total amyloid burden and Aβ1-40, compared to either alone, but no difference in Aβ1-42, tau, or pathologic tau. Interestingly, the index case had similar IBA1 measures to the DS group, suggesting potential proximal relationships between trisomy 21-driven AD and neuroinflammation.
Conclusions: This unique research brain donor allows for comparison of AD neuropathologic burden in combined DS and a PSEN2 variant to DS and PSEN2 alone. Overall, the study presents an opportunity to identify potential common pathways, critically distinct mechanisms, and points of interaction through quantitative neuropathological analyses, which may inform future experimental models in DS and FAD.

PLATFORM 2: Tumors: Glial

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Can variant allelic frequencies of driver mutations predict false-negative MGMT pyrosequencing results in adult-type diffuse gliomas?

M McCord[1], P Jamshidi[1], V Thirunavu[1], L Santana-Santos[1], J Walshon[1], K McCortney[1], A Steffens[1], E Vormittag-Nocito[1], L Jennings[1], K Galbraith[2], H Zhang[1], R Lukas[1], R Stupp[1], P Kumthekar[1], A Heimberger[1], M Snuderl[2], C Horbinski[1]
[1]Northwestern University; [2]New York University

Background: MGMT promoter methylation predicts favorable temozolomide (TMZ) response in gliomas. Accurate test results depend on adequate tumor cellularity in analyzed samples, which is usually estimated (with sub-optimal accuracy) via light microscopy. We evaluated driver mutation variant allelic frequency (VAF) as a quantitative metric for tumor cellularity in MGMT promoter methylation testing.
Methods: In a cohort of 692 adult-type diffuse gliomas, MGMT promoter methylation was tested by either pyrosequencing or DNA methylation array. VAF of TERT and IDH driver mutations was quantified by next generation sequencing (NGS). We compared frequency of methylated (positive) and unmethylated (negative) results (Fisher's exact test) and promoter methylation levels (unpaired t-test) according to VAF.

We correlated MGMT promoter methylation, VAF, visually estimated tumor cellularity, and cellularity inferred from VAF via linear regression. We assessed survival (Log-Rank test) for 241 patients with IDH-wildtype GBM treated with TMZ.

Results: In samples with VAF< 28.5% (identified by Cutoff-Finder), pyrosequencing yielded fewer positive results (44.0% versus 66.0%, $p < 0.0001$) and lower methylation levels (11.9% versus 18.6%, $p < 0.0001$) than samples with VAF≥28.5%. Methylation array showed less frequent positive results at low VAF for IDH-mutant astrocytoma, but not for IDH-wildtype GBM. Methylation level by pyrosequencing correlated with VAF (R^2=0.039). Visual cellularity estimates correlated best with VAF-based cellularity when VAF was high, but diverged when VAF was low (R^2=0.558). GBMs with unmethylated MGMT by pyrosequencing and TERT VAF< 10.0% showed surprisingly favorable responses to TMZ versus cases with VAF≥10.0% (median survival=35.1 versus 15.8 months, $p = 0.046$). Twelve GBM pyrosequencing samples were re-tested by DNA methylation array. Results changed for 2/6 samples with TERT VAF≤10%, and 0/6 samples with VAF>10%.

Conclusions: Driver mutation VAF is useful for quality assurance in MGMT promoter methylation testing. Tumor samples with low VAF are at greater risk of false-negative results by pyrosequencing, which can be corrected by DNA methylation array testing.

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Spatial Transcriptomic Profiling of Hypercellular Nodules Reveals Activation of Cholesterol Biosynthesis and Synaptogenesis Pathways

G López[1], M Liu[1], K Abramson[1], E Hocke[1], S Hilz[2], J Lee[3], G Zhang[1], J Costello[2], R McLendon[1], D Ashley[1], S Gregory[1]
[1]Duke University; [2]University of California, San Francisco; [3]Oregon Health Sciences University

Background: Oligodendrogliomas represent approximately 5-20% of gliomas. Hypercellular nodules (HCN) are present in 9-17% of cases and demonstrate increased mitotic activity and nuclear atypia; however, the signaling pathways driving these features remain unknown.

Methods: We performed GeoMX Digital Spatial Profiling (DSP, Nanostring) in 10 oligodendrogliomas with HCN using a panel of 89 proteins. We performed spatial transcriptomics on six oligodendrogliomas with HCN (10x Genomics Visium).

Results: By DSP, we identified an increase in NFIB (Nuclear Factor 1B) expression within HCN (fold-change (FC, log2) = 1.0, p-value = 0.054). These data suggested that HCN may represent regions of increased stemness. To assess gene expression changes at the whole RNA transcriptome level, we performed spatial transcriptomic analysis, which assesses the whole transcriptome within 55 um regions on the slide. Analyses revealed a decrease in RNA expression of markers of differentiation within the HCN, including GFAP and MBP, substantiating the spatial protein analyses. Unsupervised clustering of the 55um regions identified nodules as distinct clusters within the tumor. Within each tumor, we compared clusters defining the nodules to remaining tumor and performed Gene Ontology enrichment analysis and Ingenuity Pathway Analysis (Qiagen) based on differentially expressed genes. Within the majority of nodules, we identified an increase in signaling through pathways involved in nervous system development, synaptogenesis, and cholesterol biosynthesis.

Conclusions: These studies provide evidence that hypercellular nodules represent regions of increased stemness with increased activity through neurodevelopmental pathways and cholesterol biosynthesis. Multi-omic analysis is ongoing, including matched single nucleus RNA sequencing for each sample and matched ATAC-seq in a subset (10x Genomics Chromium). These studies will allow for deconvolution of the spatial transcriptomic data and determination of the tumor cell types that predominate within hypercellular nodules. By identifying pathways activated in these regions of anaplasia and increased mitotic activity, these studies identify new potential avenues for therapeutics in oligodendroglioma.

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Prognostic value of DNA methylation, aneuploidy, and CDKN2A/B homozygous deletion in predicting clinical outcome of IDH mutant astrocytomas

K Galbraith, M Garcia, C Schroff, J Serrano, I Tran, M Snuderl
NYU Langone Health

Background: IDH mutant astrocytoma grading, until recently, has been morphology based. The 5th edition of the Central Nervous System WHO introduces CDKN2A/B homozygous deletion. The DNA methylation classifier identifies two epigenetic subclasses of IDH astrocytoma. We sought to investigate prognostic impact of molecular biomarkers.

Methods: We analyzed 98 IDH mutant astrocytomas diagnosed at NYU Langone Health between 2014 and 2022. We analyzed DNA methylation subclass, CDKN2A/B homozygous deletion, and ploidy. We correlated molecular biomarkers with histological grade, progression free (PFS) and overall (OS) survival, and outcome.

Results: Our cohort included 39 WHO grade 2, 31 WHO grade 3, and 28 WHO grade 4 IDH mutant astrocytomas. Tumors were stratified into two groups by DNA methylation, astrocytoma IDH low grade (A_IDH, N = 58) and astrocytoma IDH high grade (A_IDH_HG, N = 40). CDKN2A/B homozygous deletion was detected in 39 cases (49%) and aneuploid complex copy number plot in 39 cases (49%). The average PFS and OS for WHO grade 2 tumors was 56 months and 144 months, for WHO grade 3 tumors was 51 months and 74 months, and for WHO grade 4 tumors was 39 months and 60 months, respectively. There was no difference in PFS based on grade, DNA methylation, CDKN2A status, or aneuploidy. Overall survival was not significantly different for grading by WHO histological criteria or by aneuploidy (P-value 0.0699 and 0.0588). However, OS was significantly different when

stratified by methylation classes (P-value=0.0016), or by CDKN2A/B status (0.0286).
Conclusions: The current WHO recognized grading criteria for IDH mutant astrocytomas show limited prognostic value. Stratification based on DNA methylation shows superior prognostic value for OS.

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Allele-specific loss or mutations in CDKN2A/B worsen overall survival in IDH-mutant astrocytomas

R Hickman[1], E Gedvilaite[2], R Ptashkin[3], R Cimera[2], C Vanderbilt[2], J Benhamida[2], T Fahy[2], R Young[2], A Miller[2], I Mellinghoff[2], M Rosenblum[2], M Ladanyi[2], M Arcila[2], R Brannon[2], Y Zhang[2], T Bale[2]
[1]Human Oncology and Pathogenesis Program, Sloan Kettering Institute, New York, NY, 10065; [2]Memorial Sloan Kettering Cancer Center; [3]C2i Genomics

Background: Homozygous deletion (HOMDEL) of CDKN2A/B predicts rapid progression with poor overall survival (OS) in IDH-mutant astrocytomas and signifies a WHO grade 4 diagnosis regardless of histologic appearance. The prognostic significance of other mechanisms of CDKN2A/B inactivation, in addition to HOMDEL, remain unclear, as does the preferred method of detection. We investigated whether non-synonymous mutations and allele-specific copy number alterations (ASCNA) of CDKN2A/B could impact OS and improve prognostication than somatic copy number alterations (SCNA) alone.
Methods: Matched tumor-normal targeted sequencing of all protein-coding exons and select introns of 341-505 genes was performed using MSK-IMPACT^{TM} of 382 IDH-mutant astrocytomas. SCNA were determined using loess normalized sequence coverage of targeted regions in each tumor sample compared with a standard diploid nontumor sample. We additionally deployed the FACETS algorithm (Fraction and Allele-Specific Copy Number Estimates from Tumor Sequencing) that discriminates alleles uses a SNP-based approach, to evaluate ASCNA across genomic targets.
Results: CDKN2A/B loss was the most frequent SCNA and linked to shorter OS than copy neutral samples (13.6% (n = 52), median OS: 1.9 vs. 12.6 years, $P < 0.01$). CDKN2A/B non-synonymous mutations were identified in 12 CDKN2A/B copy neutral tumors and associated with shorter OS than wildtype tumors (1.6 vs. 11.8 years, $P < 0.01$). Tumors with CDKN2A/B heterozygous loss (HETLOSS) lacking a CDKN2A/B mutation had intermediate survival (median OS: 6.3 years) between CDKN2A/B intact tumors (median survival: 15.4 years, $P < 0.01$) and CDKN2A/B HOMDEL/mutant tumors (median OS: 2.1 years, $P < 0.01$). Additional gains in CCND2 and/or CDK4 (CDKN2A/B-related RB pathway genes) worsened OS in HETLOSS tumors (median OS: 1.4 years, $P = 0.01$).
Conclusions: Genetic alterations in CDKN2A/B, besides HOMDEL, associate with shortened OS. Detailed analysis of CDKN2A/B alterations and related RB pathway genes through tumor-matched normal sequencing data enhances prognostication of IDH-mutant astrocytomas beyond existing guidelines.

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Clinicopathologic analysis of novel subtypes of adult-type IDH-wildtype diffuse high-grade gliomas

M Nasrallah[1], D Zhang[1], O Singh[2], Z Abdullaev[2], F Andreiuolo[3], E Borys[4], S Camelo-Piragua[5], B Christensen[6], H Colman[7], F D'Almeida Costa [8], C Eberhart[9], C Giannini[10], J Helgager[11], R Li[12], I Lima Fernandes[13], G Lopez[14], R Macaulay[15], J Mandell[16], J Mendez[17], L Neder Serafini[18], S Neill[19], A Nguyen[20], S Nix[21], K Phillips[22], P Pytel[23], A Silveira De Souza[24], E Sloan[25], M Smith-Cohn[26], J Trejo-Lopez[27], R Turakulov[2], K Aldape[2]
[1]University of Pennsylvania; [2]Center for Cancer Research, National Cancer Institute, National Institutes of Health; [3]D'Or Network Hospitals and Institute for Research and Education (IDOR), and Paulo Niemeyer State Brain Institute, Rio de Janeiro, Brazil; [4]Loyola Pathology; [5]University of Michigan; [6]Dartmouth Geisel School of Medicine; [7]Huntsman Cancer Institute, University of Utah; [8]A.C.Camargo Cancer Center/DASA; [9]Johns Hopkins University; [10]Mayo Clinic, Rochester, MN and Alma Mater Studiorum University of Bologna (Italy), [11]University of Wisconsin School of Medicine and Public Health; [12]Children's of Alabama; [13]Laboratório Bacchi, Sao Paulo, Brazil; [14]Duke University; [15]Moffitt Cancer Center, Tampa, FL; [16]University of Virginia; [17]Huntsman Cancer Institute/University of Utah; [18]University of São Paulo; [19]Emory University School of Medicine; [20]Mayo Clinic, Rochester, MN; [21]UAMS Health; [22]The Ben and Catherine Ivy Center for Advanced Brain Tumor Treatment at Swedish Neuroscience Institute, Seattle, Washington; [23]University of Chicago; [24]Rede D'Or / D'Or Institute Research & Education (IDOR), Rio de Janeiro, Brazil; [25]MedStar Georgetown University Hospital; [26]Benefis Health System / Sletten Cancer Institute; [27]Mayo Clinic, Rochester, Minnesota

Background: Methylation profiling of brain tumors has revealed novel subtypes determined by methylation signature grouping, and the biological and clinical significance of some of these novel subtypes is unknown. Among the novel groups are three entities termed by the 12.5 version of the Heidelberg brain tumor methylation classifier as adult-type diffuse high grade glioma, IDH-wildtype, subtype B (HGG_B), subtype E (HGG_E), and subtype F (HGG_F).
Methods: We have identified 75 cases belonging to these groups: HGG_B (22 cases, 36% female), HGG_E (31 cases, 52% female), and HGG_F (22 cases, 32% female), and assess clinical, radiologic, histologic and molecular features of these cases.
Results: Although the Heidelberg descriptor names these classes "adult-type," 52% of HGG_E are pediatric, with a median age at diagnosis of 11 years. The histology varies among methylation classes; HGG_B cases demonstrate high-grade and predominantly astrocytic morphology, HGG_E cases exhibit

high-grade glial histology with distinct nuclear features, and HGG_F cases show lower grade glial morphology. MGMT promoter methylation differs across the HGG_B (0%), HGG_E (23%), and HGG_F (14%) subtypes. Mutational analyses are in progress, but to date show enrichment of TERT promoter mutations in the HGG_F group, while HGG_E samples are enriched for tumors with POLE mutations and elevated tumor mutation burden.

Conclusions: Our work represents an initial effort to understand the morphologic, genomic and clinical characteristics of these currently uncharacterized subtypes of gliomas.

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The clinical and molecular landscape of gliomas in adolescents and young adults

C Hawkins[1], J Bennett[1], S Das[2], A Gao[3], J Keith[4], M Lim Fat[4], D Munoz[2], L Nobre[1], A Sahgal[4], D Tsang[3], G Zadeh[3], U Tabori[1]

[1]The Hospital for Sick Children; [2]Unity Health; [3]University Health Network; [4]Sunnybrook Health Sciences Centre

Background: There have been extensive studies of the molecular and clinical features of glioma, however gliomas in adolescents and young adults (AYA), representing the crossroads between pediatric-type and adult-type glioma remain an orphan population. To address this we assembled a population-based cohort of AYA glioma to determine the relative prevalence and clinical significance of molecular alterations in these patients.

Methods: Patients aged 15-39.9 years diagnosed with glioma at University of Toronto Medical Centers from 2000-2019 were eligible. Comprehensive molecular analysis was performed and therapeutic and outcome data collected.

Results: A total of 876 AYA gliomas were identified. Genetic alterations were found in 95% of available tumors. Pediatric-type mutations were found in 33% of AYA gliomas with relative frequencies different from those seen in pediatric cohorts. Most commonly found were BRAF p.V600E (11%) and FGFR alterations (7%) while BRAF fusions (4%), H3 p.K27M (4%) and H3.3 p.G34R (1%) were rare. IDH mutation was found in 57% of tumors with GBM accounting for 7%. Pediatric-type gliomas had different outcomes in AYA than in children. Ten-year OS of 100%, 90% and 95% was seen for BRAF fused, BRAF-V600E and FGFR-altered AYA low grade glioma (LGG), compared to 14% and 25% for BRAF-V600E and FGFR-altered high grade glioma (HGG) respectively. BRAF and FGFR mutant tumors had higher proportion of HGG versus LGG in AYA compared to children (OR 2.6, 95% CI 1.2-5.6). Surprisingly, outcome was better for AYA LGG compared to childhood LGG with a 10 year PFS of 76.8% vs 51.6% respectively ($p = 0.0009$). This suggests a "window of opportunity" for progression exists and more indolent disease in those presenting with LGG as AYA.

Conclusions: AYA gliomas are enriched for pediatric-type alterations with distinct outcomes. Routine analysis is required given the role for targeted inhibitors.

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DGONC comprise three molecular subtypes with unique DNA methylation and copy number profiles and overlapping histopathology

D Thomas[1], K Schieffer[2], O Singh[3], S Ramadesikan[2], M Lazow[2], D Pratt[3], Z Abdullaev[3], M Deng[4], D Jones[4], F Sahm[5], M Quezado[3], C Ketchum[3], D Marker[6], S Kulich[6], C Pierson[2], D Boue[2], M Fouladi[2], J Leonard[2], C Cottrell[2], K Aldape[3]

[1]Nationwide Children's Hospital; [2]Nationwide Children's Hospital; [3]NIH; [4]Hopp Children's Cancer Center; [5]German Cancer Research Center; [6]UPMC

Background: Diffuse glioneuronal tumor with oligodendroglioma-like features and nuclear clusters (DGONC) is a molecularly defined tumor type that emerged from genome-wide DNA methylation profiling studies. DGONC shows variable histologic differentiation that overlaps with other central nervous system tumors. Monosomy 14 is a recurrent alteration, although not unique to DGONC. A molecular driver mutation or fusion has not been identified.

Methods: In this study, we characterized a cohort of 22 DGONC confirmed by DNA methylation profiling using the Heidelberg classifier. Our cases were analyzed together with 31 DGONC cases previously reported by Deng et al.

Results: Monosomy 14 is frequent in DGONC but not universal (44/53; 83%). Unsupervised clustering analysis of methylation data from the combined cohort indicates DGONC is comprised of three molecular subgroups with unique methylation and copy number profiles. Nearly all cases in subgroups 1 (9/9; 100%) and 2 (25/27; 93%) possess monosomy 14, while only 59% of cases in subgroup 3 have loss of chromosome 14 (10/17). Overall, subgroup 1 is mostly copy neutral except for monosomy 14, while subgroups 2 and 3 have other copy number alterations including gains at chromosome 8p, 8q, 17p, 17q as well as loss of 3p. Gain of chromosome 2p and 2q appears unique to subgroup 3 (60%). Within each subgroup, DGONC may show traditionally low-grade or high-grade histologic features with regard to cellularity, cytologic atypia and mitotic activity. We identified a significant association of subgroup 1 tumors with low-grade histologic features (89%) while tumors in subgroup 2 exhibited more high-grade features (70%).

Conclusions: We hypothesize the unique methylation and copy number profiles of the different DGONC subgroups contribute to the variability in histologic features. Consistent with prior reports, our limited outcome data suggest DGONC may be associated with a better prognosis than high-grade gliomas or embryonal tumors.

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Epidemiologic, Histologic, and Molecular Features of Methylation Class Pleomorphic Xanthoastrocytoma

C Dampier[1], Z Abdullaev[1], K Aldape[1], K Galbraith[2], C Giannini[3], C Ketchum[4], D Pratt[1], M Quezado[1], S Rajan[1], N Shah[1], M Snuderl[2], R Vaubel[3]

[1]National Cancer Institute; [2]New York University; [3]Mayo Clinic; [4]University of Illinois

Background: Genome-wide DNA methylation profiling is a valuable complementary method of CNS tumor classification. Methylation class pleomorphic xanthoastrocytoma (mcPXA) comprises tumors with the same methylation signature as classical PXA, but the clinical relationship between mcPXA and classical PXA remains uncertain.
Methods: To help resolve this uncertainty, we examined epidemiologic, histologic, and molecular features of a large cohort (n = 428) of tumors matching to mcPXA by the DKFZ classifier and unsupervised clustering.
Results: Patient median age was 21 (range 1-73) with a female predominance (237 female/191 male). Most (n = 316, 74%) received DKFZ v12 confidence scores > =0.85. In the subset of cases available for histologic review (n = 160), classical morphology was present in many but not all and was not associated with confidence score (pleomorphism n = 127; xanthomatous cells n = 90; granular bodies n = 92). CDKN2A/B homozygous deletion was observed in 343 (80%) cases. In the subset of cases tested for BRAF p.V600E (n = 167), 143 (86%) harbored the mutation. Concomitant chr7+/chr10- was observed in 90 (27%) cases. In the subset of cases tested for TERT promoter mutations (n = 80), 21 (26%) harbored the mutation, frequently with co-occurrence of BRAF p.V600E (20/21) and infrequently with chr7+/chr10- (4/21). Among diagnoses rendered prior to methylation-based classification, PXA was the most common (n = 106) followed by glioblastoma (n = 86). Analyses of cases with survival data showed that TERT promoter mutation was associated with shorter overall survival (p = 0.035, as previously reported), and chr7+/chr10- was associated with shorter progression free survival (p = 0.047).
Conclusions: In summary, mcPXA includes tumors with molecular and morphologic features of classical PXA but also includes tumors without classical PXA morphology. Further characterization of clinical outcomes will help clarify the relationship between morphologic PXA and mcPXA tumors.

PLATFORM 3: Developmental/Pediatric and Muscle

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Developing hindbrain motor neurons show spatial and temporal transcriptomic diversity mapping to wiring decisions

M Rose[1], T Ray[2], N Shirooni[1], A Tenney[2], D Creighton[2], A Gelber[2], E Abdollahzadeh[1], A Barroga[1], K Chen[1], H Guardiano[1], J Li[1], L Nguyen[1], V Nguyen[1], J Padilla-Trigo[1], B Pham[1], Y Trinh[1], X Yang[1], M Tischfield[2], E Murray[3], T Collins[2], A Nugent[2], P Ang[2], S Izen[2], M Bauer[2], W Huang[2], R Satija[4], O Rozenblatt-Rosen[3], E Macosko[3], F Chen[3], A Regev[3], E Engle[2]
[1]University of California Irvine; [2]Boston Children's Hospital; [3]Broad Institute; [4]New York Genome Center

Background: The brainstem ocular motor neurons (OMNs) mediate eye movements and are differentially affected in some disorders, compared with other motor neurons (MNs). In congenital cranial dysinnervation disorders (CCDDs) such as Duane Syndrome, OMN subpopulations show disrupted or aberrant innervation, while in Amyotrophic Lateral Sclerosis (ALS), OMNs continue to function while other MNs degenerate. Here we define unique gene expression patterns among developing MNs, and generate a toolbox of protocols and genetic markers to help study these disorders.
Methods: We combine various mouse genetic reporter lines with intersectional temporal and spatial transcriptomics (bulk-, single cell-, and single nuclei RNA-seq, and Slide-seq) to isolate and compare eight distinct mouse MN populations from embryonic days E9.5-E18.5: the three ocular motor nuclei (CN3, CN4, CN6) and the other primary MN types (CN5, CN7, CN9/10, CN12 in brainstem, and spinal MNs). Gene expression was validated with database analysis, in situ hybridization, antibodies, and genetic axonal labeling. We correlate gene expression differences with cell age by both EdU labeling and tamoxifen-mediated temporal CreER induction, and visualize iDISCO- and EyeDISCO-cleared whole embryos by light sheet microscopy.
Results: Each MN population shows a unique genetic fingerprint, including novel markers of spatially- and temporally-distinct OMN subpopulations. Some OMN nerve branches correspond with cell birthdate and selectively contribute to specific aberrant branches in the Mafb-knockout mouse model of Duane Syndrome.
Conclusions: Overall, this MN transcriptomic atlas uncovers distinct developmental gene expression patterns and markers of the various cranial motor neurons, and provides new tools to study their differential vulnerability in the CCDDs and other motor neuron disorders.

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Perinatal hypoxic-ischemic brain injury: What's behind the "Ribbon Effect"?

A Viaene[1], E Nelson[2]
[1]Children's Hospital of Philadelphia; [2]The Office of Chief Medical Examiner of the City of New York

Background: The perinatal period is characterized by unique patterns of brain injury not observed outside specific timepoints in development. One example is the perceived color reversal of the gray and white matter, characterized by a pale cortex and diffusely dusky underlying white matter. This macroscopic finding was termed "ribbon effect" by Larroche in 1977 and is thought to be indicative of hypoxic-ischemic injury. However, the clinical and microscopic correlates to this macroscopic finding have not been clearly defined.
Methods: We performed an institutional retrospective study of autopsies from 2002 to 2022. Of 1,915 brain autopsies (range 14 weeks gestation-34 years), 190 were noted to exhibit ribbon effect. Cases with described ribbon effect and age- and sex-matched controls were selected for further study including blinded scoring of microscopic injury and vascular congestion.

Results: Ribbon effect was seen in subjects ages 20 weeks gestation to 9.5 months adjusted age (mean 2.0 weeks for liveborn and 36 weeks gestation for stillborn). Associated clinical findings included placental (33%), cardiac (27%), and pulmonary (18%) abnormalities and infection (13%). Imaging findings included no abnormalities (44%), white matter changes (22%), hemorrhage (21%), and hydrocephalus (18%). Degree of white matter vascular congestion significantly correlated to macroscopic severity of ribbon effect. Degree of white matter gliosis and severity of acute neuronal injury were not significantly correlated to ribbon effect. A variety of histologic findings were seen in cases with ribbon effect including focal acute neuronal injury (92%), diffuse white matter gliosis (66%), periventricular white matter necrosis (30%), white matter calcification (22%), and pontosubicular necrosis (11%).
Conclusions: These findings confirm ribbon effect is unique to the perinatal period, prior to appreciable myelination, and is seen in various clinical settings. Varied hypoxic-ischemic changes are present in nearly all cases; however, the presence of ribbon effect does not predict microscopic findings apart from vascular congestion.

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Reconstructing Human Astrocyte Lineages and Developmental Drivers with Single Nucleus Transcriptomics and Epigenomics

N Tsankova[1], S Ramos[1], B Giotti[1], Z Mussa[1], A Tsankov[1], E Falk[2]
[1]Icahn School of Medicine at Mount Sinai; [2]Boston Children's Hospital

Background: Human gliogenesis begins during the second trimester and accelerates greatly during the third trimester of gestation, in line with cortical expansion. With the advent of single cell multiomics, our understanding of both neuronal and oligodendroglial lineage specification has been greatly expanded. In contrast, the dynamics of astrocyte development and lineage specification remains much less well understood.
Methods: Recently, our group generated a comprehensive single-nucleus RNA sequencing (snRNA-seq) atlas of middle-to-late prenatal cortical development, derived from the proliferative germinal matrix and laminating cortical plate of 15 prenatal, non-pathological postmortem samples at 17-41 gestational weeks, capturing gliogenesis with high spatiotemporal resolution (Ramos et al, Nature Commun 2022). Here, we expand this snRNA-seq dataset to also include early postnatal samples dissected from the germinal matrix (3 and 6 weeks of postnatal age).
Results: Integration and cluster annotation of prenatal and postnatal samples enabled us to capture maturing protoplasmic and fibrous astrocyte cell types. Using computational tools, including lineage trajectory and RNA velocity, we infer two distinct pathways of astrocyte lineage specification: direct (arising from PDGFRB+ radial glia-like precursors) and indirect (transitioning through a transient EGFR+ glial intermediate progenitor cell type, gIPC). Regulon analysis of prenatal snRNA-seq prioritized enrichment of the FOXO1 pioneer transcription factor (TF) in astrocyte-committed gIPC-A. To access how open chromatin changes during astrogenesis and to infer TF activity from motif accessibility, we generated a parallel chromatin accessibility dataset, snATAC-seq, from matching prenatal and postnatal samples. Ongoing multiomic analyses using integrated snRNA-seq and snATAC-seq datasets allow us to interrogate further uniquely upregulated and differentially accessible TFs as putative drivers for each lineage of astrogenesis.
Conclusions: Understanding how open chromatin changes as astrocytes mature and become post-mitotic, and uncovering the underlying TF drivers associated with these changes, will provide context for future differentiation therapy in various disease states, including in glioblastoma.

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Cortical excitatory neuronal Dyrk1a trisomy disrupts juvenile visual system plasticity in a mouse model of Down syndrome

C William, M Ramani, X Pei
New York University School of Medicine

Background: The identification of genes responsible for developmental delay in Down syndrome (DS) is critical for the design of therapies to ameliorate impairment in juvenile patients. The Ts65Dn DS mouse model demonstrates defects in ocular dominance plasticity (ODP), a juvenile visual cortical response to monocular deprivation (MD) that results in strengthening and widening of cortical responses to nondeprived eye stimulation. The Ts1Rhr line, a model trisomic for about 33 genes, has a similar phenotype, suggesting trisomy of at least one gene in this small duplication impairs cortical plasticity. We have assessed the role of the candidate gene dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1a), in ODP impairment in Ts1Rhr trisomic mice.
Methods: Ts1Rhr trisomic mice and Ts1Rhr;Dyrk1a+/- mice (Ts1Rhr, Dyrk1a disomic mice) underwent 6-d MD beginning at P27-29. Cortical responses were measured by awake optical imaging of intrinsic signals and the width of responsive visual cortex ipsilateral to the nondeprived eye was measured by post mortem Arc in situ hybridization. These assays were also performed in Ts1Rhr mice heterozygous for a floxed Dyrk1a allele and an allele of either a cortical excitatory (Emx1-Cre) or pan-inhibitory (Gad2-Cre) Cre-driver, and the results compared to Cre-negative littermate controls.
Results: Dyrk1a disomy rescued ODP in Ts1Rhr mice (Ts1Rhr post-MD Ocular Dominance Index, ODI, 0.11 ± 0.04; Ts1Rhr; Dyrk1a+/- post-MD ODI, -0.133 ± 0.08; $p = 0.009$, t-test; Ts1Rhr responsive domain width 977 ± 88 microns; Ts1Rhr; Dyrk1a+/- domain width 1050 ± 98 microns; $p = 0.002$, t-test). Restoration of disomy in Ts1Rhr cortical excitatory neurons also rescued plasticity (Ts1Rhr; Dyrk1a+/flox post-MD ODI, 0.028 ± 0.043; Ts1Rhr;Emx1-Cre;Dyrk1a+/flox post-MD ODI, -0.221 ± 0.080; $p = 0.02$, t-test, $n = 7$-8 per group), while restoration of disomy in inhibitory neurons (Ts1Rhr;Gad2-cre;Dyrk1a+/fl mice) did not (data not shown).

Conclusions: These data suggest that Dyrk1a trisomy in excitatory neurons impairs cortical plasticity in Ts1Rhr mice. The identification of drug-targetable processes dysregulated by Dyrk1a trisomy may reveal therapeutic approaches to ameliorate impairment in the juvenile DS brain.

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Aplasia of the Dentate Gyrus: Report of 2 Pediatric Cases

A Gupta[1], S Saxton[2], Y Tang[2], R Folkerth[2]
[1]Office of Chief Medical Examiner, New York City; [2]New York City Office of Chief Medical Examiner

Background: Neuropathologic reports of absence of the hippocampal dentate gyrus (DG) are rare, to our knowledge, comprising an isolated finding in a single report in an octogenarian (PMID: 17001817).
Methods: From our case files, we report 2 cases in which complete neuropathologic analysis detected DG aplasia, among other notable neurodevelopmental abnormalities.
Results: Case 1, aged 2 years, carried a clinical diagnosis of "Dandy-Walker malformation", seizures since birth, and obstructive sleep apnea, requiring gastric tube and continuous positive airway pressure therapy. She was found unresponsive after a sleep period and a few-day history of respiratory symptoms. At autopsy, she had human rhino/enterovirus-positive bronchopneumonia, as well as micrencephaly, pontocerebellar hypoplasia (resembling PCH2 or PCH8), DG aplasia, status marmoratus and white matter volume loss. Case 2 was 18 years old, nonverbal, with "cerebral palsy" and seizures since birth. Whole-exome sequencing had detected several variants of unknown significance in SCN7A, GOSR2, and LLGL1. He was found unresponsive after a presumed seizure. Cerebral dysgenetic findings included temporal pachygyria, complex frontoparietal gyration focally associated with a venocapillary malformation (FCD IIIC), hippocampal dysgenesis with DG aplasia, callosal agenesis, periventricular nodular heterotopia, and pontocerebellar hypoplasia.
Conclusions: Our cases share the commonalities of epilepsy, intellectual disability, DG aplasia and pontocerebellar hypoplasia, and somewhat resemble patients with AUTS2 syndrome (intellectual disability, "cerebral palsy", epilepsy, microcephaly, and abnormal facies; PMID: 23332918), but in whom the neuropathology has not been described. Moreover, they resemble Auts2 animal models which have defects in cell proliferation (microcephaly), small or deformed cerebellum, and small dentate gyrus (PMID: 23447624). We therefore speculate whether variants in AUTS2 or related genes could lead to the rare phenotype of DG aplasia.

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Cylindrical spirals of skeletal muscle in patients with neurological diseases

J Lu, J Fournier, G Mak, K Gordon, J Glogauer, F Fareez, J Provias, M Tarnopolsky, J Lu
McMaster University

Background: Cylindrical spirals (CSs) are ultrastructurally distinct, intracytoplasmic inclusions characterized by concentrically wrapped lamellae, sometimes associated with tubular aggregates. They are rarely seen on electron microscopy (EM) in skeletal muscle biopsies with approximately 20 cases reported over the past decades. CSs are often confused with other EM concentric structures, including concentric laminated bodies (CLBs) and mitochondrial concentric cristae (MCC) due to similarities among these ultrastructures. However, CSs are distinguishable from CLBs by the presence of trilamellar ultrastructures with double electron-dense membranes of each lamella, and often connecting or coexisting with tubules; from MCC by the absence of mitochondrial residua. The clinicopathological significance of CSs remains to be elucidated.
Methods: We searched the pathology database (2012-2022) for CS, CLB, or MCC from 1671 muscle biopsies with EM examination.
Results: This study revealed CSs in muscle biopsies (8 vastus lateralis and 1 deltoid; 0.5%) from 9 patients (ages: 1-74 years; median 48 years; 1 male/8 female). These patients had neurological diseases including atypical amyotrophic lateral sclerosis, Huntington's disease, MELAS (Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes), and other complex neurological disorders with neuropathy or encephalopathy, as well as anti-MDA5+ dermatomyositis. Eight of 9 patients (except one with dermatomyositis) had genetic abnormalities such as PMP 22 gene deletion, trinucleotide repeat expansion of huntingtin gene, ALS2 variant, and m.3243A>G mutations. On histological/histochemical and EM examinations, we identified CSs in all 9 muscle biopsies to varying amounts ranging from segregated and sparse to focally frequent and aggregated; associated muscle fiber denervation atrophy in all 9 cases, as well as other associated features including tubular aggregates, mitochondrial paracrystalline inclusions and MCC.
Conclusions: Our findings suggest that CSs may be highly variable in frequency and likely underreported without EM examination routinely on muscle biopsies; they are commonly associated with neurogenic myopathy or central/peripheral nervous system disorders including some genetic neurological/neuromuscular diseases.

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Ischemic Myopathy in the Leg Muscles of Patients with Peripheral Artery Disease: Autophagy Fails to Respond to the Increased Damage

S Li, M Schieber, C Shields, K Brunette, Z Zhu, A Hakim, J Kim, P Mishra, G Casale, I Pipinos
University of Nebraska Medical Center

Background: Peripheral Artery Disease (PAD) affects over 200 million patients globally. Atherosclerotic blockages in the leg arteries of PAD patients produce a myopathy in the ischemic leg skeletal muscles, characterized by oxidative damage (OxD) and mitochondria damage. Cells depend on (Mar-

co)autophagy to remove permanent OxD proteins and organelles and mitophagy to remove damaged mitochondria. However, studies of autophagy in PAD are very limited. This project is the first to quantitatively evaluate autophagy in PAD and its relation to PAD myopathy and PAD progression.
Methods: Twelve controls, 20 early-stage PAD, and 14 end-stage PAD patients were recruited. Needle biopsies of gastrocnemius were performed. Samples were evaluated by quantitative Immunofluorescence microscopy for 1)autophagy (LC3), 2)mitophagy (PINK1/ATP5A ratio), and 3)OxD (Carbonyl). Cell membranes were stained with WGA for myofiber identification and morphology. LC3/Carbonyl ratios were calculated to reflect the response of autophagy to damage. Intergroup differences and intragroup correlations were examined.
Results: Myofiber Carbonyl was higher in early-stage PAD than control (2314±782 vs. 1401±436 gsu, $p < 0.005$), but severe myopathy with pathological myofiber shape/size/pattern produced wide variations in end-stage PAD samples (1742±617 gsu). Compared to control, LC3/Carbonyl (0.21±0.07 vs. 0.31±0.07, $p < 0.05$) and PINK1/ATP5A (0.53±0.15 vs. 0.78±0.30, $p < 0.05$) ratios were lower in early-stage PAD. Furthermore, positive intragroup associations between LC3 and Carbonyl were observed in control ($r = 0.48$, $p < 0.05$) and early-stage ($r = 0.40$, $p < 0.05$), but became negative in end-stage PAD ($r = -0.3626$, $p = 0.08$) suggesting that autophagy gradually fails to respond to OxD as PAD progresses.
Conclusions: Our study shows, for the first time, that autophagy and mitophagy fail to respond and clear the increased damage in the ischemic leg muscles of PAD patients and may be an essential pathophysiologic mechanism in PAD and a contributor to PAD progression. Enhancing autophagy in early-stage PAD may represent a future therapeutic target for the myopathy and symptoms of PAD patients.

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Synthetic Data Facilitates Deep Learning for Accurate and Robust Myofiber Segmentation with Limited Data

J Nirschl, H Vogel, S Yeung
Stanford University

Background: Muscle fiber histomorphometry is an important tool for diagnosing and researching muscle and nerve disorders. Quantitative histomorphometry supports the morphologic diagnosis of myopathies with fiber-type disproportion (e.g., congenital fiber-type disproportion) in clinical samples, and it is used to study muscle cell biology, development, regeneration, and pathophysiology in research settings. However, the vast majority of automated methods for myofiber segmentation are trained on immunofluorescence images, which restricts their utility in clinical practice where routine stains such as H&E, trichrome, or enzyme histochemistry are more common. The few studies on automated myofiber segmentation in H&E images rely on small datasets of mouse tissue, only apply to H&E images, and may not generalize well to clinical samples.
Methods: Images from human muscle biopsies were used to generate a large dataset of synthetic myofibers across eight histological stains (i.e., H&E, trichrome, NADH, myosin enzyme histochemistry, etc.). The synthetic images share intensity and texture characteristics with real images and have dense ground truth annotations for training deep neural networks. We use pre-training on synthetic data to reduce the need for large, manually labeled datasets and fine-tune on smaller datasets of real images. We train, evaluate, and compare the myofiber segmentation accuracy of multiple deep-learning architectures, including UNet, DeepLabV3+, and SegFormer.
Results: Pre-training with large synthetic datasets results in highly accurate muscle fiber segmentation in real images and reduces the need for manual annotations. The quantitative measurements from automated analysis are accurate and similar to manual analysis. Overall, we show how domain-specific synthetic data allows the training of deep neural networks for unique stains and tasks where manually labeled data may be limited or time-consuming to acquire.
Conclusions: Models pre-trained on synthetic data perform well with few manually labeled real images. Deep learning can help automate quantitative histomorphometry in clinical workflows and advance research in neuromuscular disorders.

PLATFORM 4: Forensics/Trauma and Vascular

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Chronic traumatic encephalopathy-like abnormalities in a routine neuropathology service - seven year follow-up

M Del Bigio, S Krawitz, N Sinha
University of Manitoba

Background: In 2016 we documented a prospective study of CTE-like neuropathologic changes in our forensic autopsy service (Noy JNEN 2016;75). We developed a flexible decision and screening algorithm to establish the validity of our findings.
Methods: From 2016-2022, cases with a history of multiple head injuries had routine brain sampling plus 4-6 additional lateral hemisphere samples. Hyperphosphorylated tau (pTau) immunostaining (AT8 antibody) was done on 5-6 sections. If 'pathognomonic' CTE-type lesions were identified in the cerebrum, additional pTau immunostaining was done for CTE staging (Bieniek JNEN 2021;80).
Results: Approximately 1800 adult brains were examined in detail. Fifty-one screened cases were negative for CTE. Sixteen cases were positive for CTE-type change (range 34-82 years, median 46.5 years; 2 female). Alcohol abuse was documented in 11/16 (3 in combination with solvent inhalation and 3 with other drugs). Other neuropathological findings included SDH, contusions, toluene leukoencephalopathy, and atrophy. Four had developmental brain anomalies. Widespread pTau deposits

(high CTE) were found in 5/16 (including an 82 year old, who also had Alzheimer pathology), but none had large neocortical pTau collections. Cases of particular interest were: 1) a male with FGFR3 mutation / hypochondroplasia who had banged his head repeatedly since childhood (bilateral retinal detachments), 2) a male with bilateral peri-Sylvian polymicrogyria of presumed genetic etiology, 3) a female with cerebral palsy and history of head banging.

Conclusions: We conclude that, in non-contact sport participants, CTE-type neuropathologic change occurs in association with repeated head trauma. Co-existing substance abuse is common, at least a determinate of risk behavior. The utility of making a diagnosis of minor CTE in this population remains to be determined. Although the 2021 working protocol is easy to use, the so-called "high CTE" category usually has minimal pTau load and seems to be disconnected from the McKee et al. 2013 stage 3/4.

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The contributions of multiple neuropathologies to cognitive symptoms in those exposed to repetitive head impacts

T Stein[1], N Saltiel[2], Y Tripodis[2], T Menzin[2], A Olaniyan[2], Z Baucom[2], R Nicks[1], V Alaverez[2], B Huber[1], J Mez[2], A McKee[1], M Alosco[1]
[1]Boston University School of Medicine; [2]Boston University

Background: Exposure to repetitive head impacts (RHI) is associated with cognitive symptoms and risk for multiple neuropathologies. The prevalence of co-morbid pathologies and contributions to cognitive symptoms in people exposed to RHI is unknown. Here, we examined the co-occurrence of 13 neuropathologies and their contributions to cognitive symptoms and dementia in RHI-exposed brain donors.

Methods: Brain tissue from 581 RHI-exposed donors was assessed for the presence of 13 neuropathologies, including Alzheimer disease (AD), Lewy body disease (LBD), chronic traumatic encephalopathy (CTE), limbic TDP-43, and cerebral amyloid angiopathy (CAA). Measures of independent functioning and cognitive difficulties were obtained from informants and medical records. Antemortem dementia was adjudicated via consensus conferences, based on informant-reported clinical presentation. Frequencies of pathological co-occurrence were compared to a simulated distribution assuming no intercorrelation, and each pathology's contribution to variance in dementia status and cognitive scale scores was determined.

Results: The sample age range was 13-97 (mean: 60.0 [20.2 SD]). Of 581 brain donors, 76.2% had at least one moderate-severe neurodegenerative or cerebrovascular pathology. The most common neurodegenerative diseases observed were CTE, TDP-43 inclusions, AD, and hippocampal sclerosis. We observed 252 unique pathology combinations, far fewer than expected ($p = 0.004$) if the pathologies were independent. The greatest contributors to dementia were AD, neocortical LBD, hippocampal sclerosis, CAA, and CTE. Altogether, all neuropathologies accounted for 48% of variance in dementia status.

Conclusions: In this sample of brain donors exposed to RHI, multiple neuropathologies were common and highly correlated. This contrasts with findings from population-based aging studies and may be a result of common exposure to RHI. Cognitive symptoms were associated with multiple neuropathologies. These findings emphasize the role of mixed neuropathologies in cognitive decline associated with exposure to RHI.

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The Nature and Progression of Neurological Disease in 20th Century Boxers

R Castellani, N Kostelecky, J Ahrendsen, P Jamshidi
Northwestern University Feinberg School of Medicine

Background: The issue of the relationship between neurotrauma and neurodegenerative disease is poorly understood. We therefore analyzed all available case material in the literature on neurological disease of 20th century boxers for better understanding. The findings could help direct efforts at identifying a traumatic encephalopathy syndrome in individuals exposed to repeated traumatic brain injury.

Methods: Publications from the 20th century that included at least a brief narrative summary of neurological disease in boxers were analyzed for neurological deficits, progressive neurological disease, and neuropathology when available (154 cases, 35 with neuropathology). Presence of progressive disease, stationary disease, and improvement in disease, were noted. Progressive disease was subdivided into progression of subcortical signs, progression of memory dysfunction, and progression to neurological failure (neurodegenerative disease-like progression). Presence of neurological signs prior to retirement, and whether neurological signs could be traced to specific fights, were noted. Each of the above were assessed independently by all co-authors. Assessments that were not unanimous were discussed and consensus was reached.

Results: The most common neurological signs reported in 20th century boxers were memory loss, dysarthria, and gait disturbances. Abnormal reflexes (often asymmetric) and vision problems were also common. Cases demonstrating typical neurodegenerative disease pathology were over-represented among those with frank dementia or progression to neurological incapacitation. Clinical presentations that were primarily psychiatric in nature, with no accompanying neurological deficit, were essentially absent from the case material.

Conclusions: Repeated traumatic brain injury from boxing resulted in permanent neurological deficits and was particularly injurious in those who fought in the early 20th century. Progressive disease varied. Typical neurodegenerative disease pathology was over-presented among those with progression to dementia. Psychiatric problems without neurological signs was not noted. These data suggest that progressive dementia and psychiatric diseases without neurological deficits might be

exclusionary in future definitions of traumatic encephalopathy syndrome.

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Neuropathologic Examination of a Large Series of Military Suicides

D Priemer[1,2], D Perl[1]
[1]Uniformed Services University School of Medicine, Bethesda, MD, USA; [2]The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

Background: Suicide rates among military personnel have steadily increased in the post-9/11 era. Though suicide is largely considered a mental health issue, factors including traumatic brain injury (TBI) and sequelae may contribute. Therefore, anatomic/organic disease may play a role in a subset of cases. The Department of Defense/Uniformed Services University Brain Tissue Repository collects donated brains from deceased military personnel, including those who die by suicide.
Methods: We reviewed 74 brains from military personnel from all Service branches who died by suicide (average age: 38.8 years [range: 20-67]). Psychiatric diagnoses were reported in 65.2% of cases, most commonly post-traumatic stress disorder. 54.9% had prior suicidal ideations and/or attempts. 52.7% of decedents had alcohol and/or substance abuse history. Blast exposure was noted in 44.4%. Impact-TBI from military activities, contact sports, and/or non-sports-related civilian activities was noted in 56.3% of cases. Deaths were most commonly by gunshot (66.7%) and hanging (22.2%).
Results: Examination revealed a mixture of pathologies. This included chronic traumatic encephalopathy in 5 cases (7.1%), all in prior contact sport athletes, and interface astroglial scarring in 9 cases (12.8%). Despite relatively young ages, 87% and 50% of cases showed evidence of chronic cerebrovascular disease and age-related tau pathology (e.g. Alzheimer's disease, primary age-related tauopathy), respectively.
Conclusions: Military suicide is a complex problem. A number of underlying factors may be involved, including organic brain pathology wherein one may presume that anatomic, biochemical and/or molecular alterations are identifiable. The availability of this brain collection provides unique opportunities toward improved understanding of biologic disturbances which contribute to suicide in the military. The information, content, and/or conclusions herein do not necessarily represent the official position or policy of, nor should any official endorsement be inferred on the part of, USU, the DoD, the U.S. Government, or the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

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Evaluating the Spatial Relationship between Microhemorrhages and Axonal Injury

R Mittenzwei[1], K Sytsma[1], B Edlow[2], D Keene[1], C Latimer[1], C MacDonald[1], C Maffei[2], H Maioli[1], D Marshall[1], A Nolan[1]
[1]University of Washington; [2]Massachusetts General Hospital and Harvard Medical School

Background: Diffuse axonal injury (DAI) is one manifestation of TBI caused by accelerative-decelerative forces inducing shearing injury within subcortical and deep white matter tracts. Clinically, susceptibility-weighted MRI (SWI) is used to identify white matter microhemorrhages, which are considered the radiographic hallmark of DAI. Microhemorrhage extent is used in clinical prognostication and decision-making; in some forensic settings, gross identification of microhemorrhages may be considered evidence of DAI without histopathologic evaluation. The dogma that radiographic and gross microhemorrhages correspond with DAI persists despite a lack of systematic pathologic studies examining this relationship.
Methods: Thus, to investigate this, three brain donors with TBI (survival interval: 7-13 days) were selected for imaging and neuropathological evaluation. Coronal brain slices were co-registered against ex vivo SWI MRI images to guide microhemorrhage sampling. All white matter microhemorrhages (defined as an SWI-dark lesion at least a voxel in size located in at least two subsequent slices at 500 microns) were sampled ($n = 39$ slides). Slides stained with hematoxylin and eosin and amyloid precursor protein (APP, axonal injury marker) underwent whole-slide scanning (Leica® Aperio AT2) and subsequent annotation and analysis using the Halo® image analysis platform (Indica Labs). Axonal injury (criteria of axonal swelling with APP staining area greater than 12.6 μm2) was assessed and analyzed as a function of distance from a microbleed by 200 micron concentric outlines up to 1mm.
Results: White matter microhemorrhages were histologically confirmed in 74% of SWI lesions. Of slides without histologic white matter microhemorrhages, 70% demonstrated congested vasculature. 65% of microhemorrhages demonstrated evidence of APP staining with morphologic axonal swellings within 1mm, and these swellings were significantly increased as a function of proximity to the microbleed. However, 35% of microbleeds did not show axonal injury.

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The spinal cord pathology is important in investigating abusive head trauma (AbHT) in children: A retrospective assessment of 40 cases

S Al-Sarraj[1], M Colombari[2], C Troakes[3]
[1]Kings College Hospital. London; [2]University of Verona; [3]The Institute of Psychiatry, Psychology and Neurosciences

Background: Abusive head trauma (AbHT) in children is a challenging diagnosis for which the current neuropathological diagnostic criteria ("the triad") of ischaemic encephalopathy (IsE), subdural haematoma (SDH) and retinal haemorrhage (RH) has been argued to be insufficient. There is increasing evidence from the Neuroradiology of additional spinal cord injury in AbHT but this is so far, it has been poorly investigated neuropathologically.

Methods: We retrospectively reviewed 110 autopsy brain examination from children (62 males and 48 females, mean age eight months) refereed for medico legal investigation over an eight-year-period. The review includes gross and histological examination of the brain and spinal cord alongside the circumstances of death and other pathological investigations. Depending on the outcome of multidisciplinary forensic experts, forty (40) are diagnosed abusive head trauma (AbHT) and nine (9) accidental head trauma (AcHT). Eight (8) cases were not clearly accidental or abusive ("undetermined" UHT) and 53 cases died from non-traumatic brain injury (NTBI).
Results: The 40 AbHT cases (1day-3years) show 100% multifocal and shallow cranial SDH 100%, IsE 100%, RH 93% and brain stem axonal injury 30%. The spinal cord shows SDH in 87%, subarachnoid haemorrhage (SAH) in 79% and spinal nerve roots axonal injury in 58% of the cases. The SDH/SAH at lumbar level was found to be significantly related to a diagnosis of abuse. When blood collection was detected at the cervical level it was consistently associated with blood collection at lower levels, suggesting a primary blood source in the spinal cord.
Conclusions: The results indicate that the spinal cord injury is common in AbHT that should be routinely examined together with the cervical spine and can be regarded as useful fourth criteria in addition to the triad in the diagnosis of abusive head trauma.

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Correlation of Pathological Findings with MRI Imaging in Traumatic Spinal Cord Injury in the Hyperacute Timeframe in a Nonhuman Primate Model

O Fayez[1], O Fayez[1], H Simmons[2], K Johnson[3], D Schalk[4], K Brunner[4], P Basu[4], S Capuano[4], S Nesathurai[5]
[1]The Shrewsbury & Telford NHS Trust; [2]Wisconsin National Primate Center, University of Wisconsin-Madision; [3]University of Wisconsin at Madison Madison WI, 53715; [4]Wisconsin National Primate Center, University of Wisconsin-Madision 1220 Capitol Ct, Madison, WI 53715; [5]McMaster University Hamilton Health Sciences St. Josephs Healthcare Hamilton 711 Concession St. Hamilton, Ontario, Canada, L9K1T1

Background: Traumatic Spinal Cord Injury (TSCI) remains a significant cause of morbidity and mortality in humans. Magnetic Resonance Imaging (MRI) has been a spectacular modality in management, however, data correlating MRI findings with pathological insults in the hyperacute time-frame (i.e., within one hour of injury) is limited. This is due to the time-period between injury and transport to hospital is typically one-hour or more. Only after assessment and stabilization can an MRI be completed. In this context, nonhuman primate models are essential to provide insights into this critical scientific hypothesis.
Methods: The subject was a Rhesus macaque. Baseline MRI imaging of the spine was obtained. A small laminotomy was performed at L5 level and an epidural balloon catheter was advanced to the level of the lower thoracic spine which was inflated rapidly and remained for one-hour to produce lesions consistent with TSCI. MRI imaging, with and without contrast, was obtained over the next hour. Subsequently, the subject was humanely euthanized and a post-mortem examination was conducted. Tissue sections were collected from the epicenter, caudal and cephalad sites of the lesion.
Results: The abnormalities were most prominent with Disco-Lava sequence MRI Technique. Sagittal images of the thoracic spine displayed increased abnormalities including increased signal intensity. The findings were consistent with edema and/or hemorrhage. Histology of coronal sections at the level of injury revealed focally extensive disruption of grey matter and central canal with marked grey matter hemorrhage, acute necrosis, and mild multifocal white matter hemorrhage. Eosinophilic material and erythrocytes were found in adjacent sections, up to 2 cm caudal to the lesion.
Conclusions: MRI abnormalities were present within one-hour after injury in acute experimental spinal cord injury. The histopathological findings are consistent with the radiological abnormalities.

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Cerebral White Matter Rarefaction has Both Neurodegenerative and Vascular Causes and May Primarily be a Distal Axonopathy

T Beach[1], C Adler[2], A Atri[1], C Belden[1], P Choudhury[1], E Driver-Dunckley[2], D Goldfarb[1], S Mehta[2], M Sabbagh[3], G Serrano[1], H Shill[3], R Caselli[4], M Cline[1], S Hemmingsen[1], A Krupp [1], A Kuramoto[1], I Lorenzini[1], M Mariner[1], K Martinez[1], R McHattie[1], S Qiji[1], S rapscak[5], A Stewart[1], C Tremblay[1], B Woodruff[4], R Arce[1], C Borja[1], M Glass[1], A Intorcia[1], L Sue[1], J Walker[1]
[1]Banner Sun Health Research Institute; [2]Arizona Mayo Clinic; [3]Barrow Neurological Institute; [4]Mayo Clinic Arizona; [5]Banner Alzheimer's Institute

Background: Cerebral white matter rarefaction (CWMR) was considered by Binswanger and Alzheimer to be due to cerebral arteriosclerosis. Renewed attention came with CT and MR brain imaging, and neuropathological studies finding a high rate of CWMR in Alzheimer's disease (AD). The relative contributions of cerebrovascular disease and AD to CWMR are still uncertain.
Methods: In 1,181 autopsies by the Arizona Study of Aging and Neurodegenerative Disorders (AZSAND), large-format brain sections were used to grade CWMR and determine its vascular and neurodegenerative correlates.
Results: Multivariable logistic regression models indicated that Braak neurofibrillary stage was the strongest predictor of CWMR, with additional independently significant predictors including age, cortical and diencephalic lacunar and microinfarcts, body mass index and female sex. It appears that while AD and cerebrovascular pathology may be additive in causing CWMR, both may be solely capable of this. The typical peri-

ventricular pattern suggests that CWMR is primarily a distal axonopathy caused by energy failure of the cell bodies of long association corticocortical projection neurons.
Conclusions: A consequence of these findings is that CWMR should not be viewed simply as "small vessel disease" or as a pathognomonic indicator of vascular cognitive impairment or vascular dementia.

FRIDAY POSTERS: Neurodegenerative: FTLD, Lewy Body, Parkinson, Other

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The First Ever Autopsy of a Patient with Brown-Vialetto-Van Laere Syndrome

M Gubbiotti, S Scherer, V Lee, E Lee
Hospital of the University of Pennsylvania

Background: Brown-Vialetto-Van Laere syndrome (BVVL) is a rare disorder with childhood onset sensory and motor deficits, as well as hearing and vision loss. BVVL is caused by recessive mutations in SLC52A2, which encodes a riboflavin transporter, and can respond to high-dose riboflavin therapy. We report the autopsy of a 61 year-old female with compound recessive mutations in SLC52A2 (p.A248D and p.G306R) who presented with symptoms consistent with BVVL at age 3 and died several decades later following complications from pneumonia.
Methods: An autopsy involving analysis of H&E stained sections of eyes, brain, spinal cord, and sural nerves was performed. Additional special and immunohistochemical stains were performed on select sections. Semi-thin sections of optic and sural nerves were assessed.
Results: Bilateral optic nerves showed severe atrophy, gliosis, and loss of myelinated axons. Near total loss of the ganglion cell layer of the retina was identified. The spinal cord demonstrated high levels of corpora amylacea and abundant microgliosis with paucity of anterior and posterior horn neurons. Myelin loss was observed within the posterior spinal columns. Semi-thin sections of nerves showed notable loss of large-caliber myelinated fibers with scattered clusters of regenerated axons. Skeletal muscle illustrated predominantly neurogenic changes. Severe hypoxic-ischemic changes were noted within all sections of the brain, secondary to ventilator support; no overt structural malformations, tumor mass, or hemorrhage were identified.
Conclusions: The findings of severe bilateral optic nerve atrophy and axonal loss, loss of the retinal ganglion cell layer, loss of spinal cord sensory and motor neurons with degeneration of the posterior spinal columns, sural nerve fibrosis with loss of myelinated axons, and skeletal muscle with neurogenic changes are consistent with the clinical symptoms observed in this patient with BVVL. As this autopsy is the first ever performed on such a patient, it provides a gold standard for future comparison.

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Neuropathological characteristics and cognitive trajectories of adults with Limbic-Predominant Age-Related Encephalopathy (LATE)

M Ospina-Romero[1], B Bendlin[2], S Salamat[1]
[1]University of Wisconsin Hospital and Clinics; [2]University of Wisconsin

Background: This study investigated the differences in neuropathological characteristics and cognitive trajectories of adults with and without limbic predominant age-related encephalopathy neuropathologic change (LATE-NC).
Methods: Data was from 79 autopsied participants with TDP-43 immunohistochemical studies from Wisconsin Alzheimer's Disease Research Center and Wisconsin Registry for Alzheimer's Prevention. We compared frequencies of common neuropathologic changes including Alzheimer's disease neuropathologic change (AD-NC), Lewy body disease, and brain arteriolosclerosis. Antemortem cognitive assessments included global clinical dementia rating, clinical dementia rating sum of boxes, and Rey Auditory Verbal Learning Test (RAVLT). Cognitive trajectories among participants with and without LATE-NC were compared using linear mixed models.
Results: Participants in our sample were 42% female with mean age at death of 77.9 years (SD 9.5). Thirty-one (39.2%) participants demonstrated LATE-NC at autopsy, with amygdala (96.8%) and entorhinal cortex (74.2%) as the most common location. Neuropathologic comorbidities more frequently found in those with LATE-NC vs without LATE-NC are high AD-NC (74.2% vs 60.4%), Lewy body disease-amygdala only (16.1% vs 10.4%), arteriolosclerosis (87.1% vs 59.6%), cerebral amyloid angiopathy (74.2% vs 64.6%), and hippocampal sclerosis (22.6% vs 2.1%). Primary age-related tauopathy (PART) was less frequent in LATE-NC group (6.5% vs 12.5%). Participants with LATE-NC demonstrated faster cognitive decline in the time before death than age-matched participants without LATE-NC.
Conclusions: Our findings confirm results from prior studies indicating higher frequency of ADNC, arteriolosclerosis, and hippocampal sclerosis in participants with LATE-NC. Differences in Lewy body disease were limited to amygdala as has been indicated in prior results. Faster cognitive decline in those with LATE-NC compared to without LATE-NC is likely explained by an increased prevalence of other neuropathologic changes.

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Beyond AD: Neuropathological and cognitive outcomes of > 80-years of age participants free of neuritic plaques in a longitudinal cohort

J Redding-Ochoa[1], M Albert[2], S Resnick[3], J Troncoso[2], A Barrett[4], K Chang[4], A Moghekar[2], T Pylyukh[5], A Yang[3]

[1]Johns Hopkins University School of medicine; [2]Johns Hopkins Medical Institutions; [3]NIA/NIH; [4]Johns Hopkins University, SOM; [5]Johns Hopkins University SOM

Background: The goal of our study is to assess the neuropathology and cognition in older individuals free of Alzheimer's disease (AD) neuropathological changes, the most common cause of dementia in the older population. In advanced age there are multiple morbidities other than AD that may have an impact in cognition as demonstrated by clinical and postmortem studies.
Methods: Using pooled data from the Johns Hopkins Alzheimer's Disease Center including participants from the Baltimore Longitudinal Study of Aging (BLSA) Cohort (n = 62) and Clinic Cohort (n = 15), we examined the neuropathological and cognitive outcomes of 77 participants, ≥ 80 years of age, whose brains were free of neuritic plaques on neuropathological assessment, i.e., CERAD 0 (average age of death: 89; men: 74%).
Results: Diffuse Aβ plaques were present in 15 brains (19%). The Braak NFT scores ranged from I to IV (96%) with the presence of neurofibrillary tangles in the medial temporal lobe close to universal (99%). Vascular lesions were present in 46 brains (60%), infarcts in 37 (48 %), hemorrhages in 4 (5%), and mixed lesions in 5 brains (7%). Lewy body pathology (LBP) was noted in 13 brains (17 %). TDP-43 proteinopathy ,i.e., LATE neuropathologic change (NC), was observed in 23% of brains with a cumulative frequency distribution indicating that LATE-NC+ increase drastically around 95 years of age. Cognitive impairment occurred in 51% of the participants, and 39% of them were fully demented at their last clinical evaluation. Logistic regression analyses showed a significant association between LATE-NC+ and MCI/Dementia (p = 0.03; OR 4.83, 95% CI). The other variables were not statistically significant.
Conclusions: In conclusion, our study indicates that even in the absence of neuritic plaques, older individuals have a high risk of cognitive impairment. This information is relevant to public health approaches to aging, dementia, and also the eventual pharmacological prevention of AD.

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The LifeAfter90 Study: 2023 neuropathology update on an ethnically diverse cohort study of oldest-old

B Dugger[1], L Jin[1], K Ortega[1], C DeCarli[1], P Gilsanz[2], D Mungas[1], C Kawas[3], M Corrada[3], R Whitmer[1]
[1]University of California, Davis; [2]2- Kaiser Permanente Northern California Division of Research; [3]University of California, Irvine

Background: Neuropathology studies of the oldest-old have significantly advanced our understanding of the multiple etiologies in very late life. Most studies include exclusively non-Hispanic White decedent; and there is a dearth of studies with ethnoracial diversity. Our goal was to characterize neuropathology in a cohort of ethnically and racially diverse oldest-old decedents.
Methods: The LifeAfter90 study is an ongoing study of Kaiser Permanente Northern California members, aged 90+ with targeted recruitment of individuals across different racial/ethnic groups with no prior diagnosis of dementia. Research assessments occur approximately every 6 months with brain donation available to all interested consenting participants. Neuropathology was assessed using the National Alzheimer's Coordinating Center Neuropathology form v.10.
Results: As of January 2023, 304 participants (40%) were enrolled in autopsy (20% Asian, 18% African American, 17% Latino, 9% Multiracial/Other, and 36% White). Of the 304 participants, 59 died and neuropathology evaluations were completed. The median age of death was 93 years (range 90-105), 33 (56%) were female, 8 Asian, 5 Black, 10 Latino, 31 White, and 5 Multiracial/Other. At final assessment, 17 participants had dementia (29%), 25 questionable/mild cognitive impairment (42%), and 16 were cognitively normal (27%). Twenty two percent of participants did not have AD, one lacked neurofibrillary tangles (NFT). High likelihood of AD was infrequent (N = 2). For vascular pathologies, 98% had some level of arteriolosclerosis, 97% had atherosclerosis, 70% had cerebral amyloid angiopathy, and 26% had at least one old microinfarct. Other pathologies were less common: 27% had Lewy bodies, 18% had TDP-43 deposits, and only one had Hippocampal sclerosis. Although data is sparse to test for associations, the proportion of individuals with dementia tended to increase with increasing pathology severity especially for AD.
Conclusions: This diverse cohort of oldest-old individuals reveal numerous brain pathologies are present with advanced age. Future analyses will investigate their role with cognitive impairment.

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Somatic genomic alterations in single neurons from brains with chronic traumatic encephalopathy (CTE)

C Ma[1], G Dong[2], S Naik[3], G McDonough[3], A McKee[4], A Huang[2], E Lee[2], M Miller[5], C Walsh[6]
[1]Harvard Medical School/Boston Children's Hospital; [2]Harvard Medical School/Boston Children's Hospital; [3]Brigham and Women's Hospital; [4]Boston University School of Medicine; [5]Harvard Medical School/Brigham and Women's Hospital; [6]Harvard Medical School/Boston Children's Hospital

Background: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disease associated with repetitive head trauma. The genetic, molecular, and cellular mechanisms behind the development of CTE are less well understood than in other neurodegenerative diseases such as Alzheimer's disease (AD). The advent of single-cell sequencing technologies allows for the study of molecular perturbations at the individual cell or cell-population level as well as the analysis of contributions of non-germline somatic mutations to disease pathogenesis. Previous studies of single-cell whole genome sequencing (scWGS) on aging and neurodegenerative brains showed that somatic SNVs (sSNVs) increase both with aging and in disease, but present with distinct patterns of muta-

tional signatures, suggesting that genetic, environmental, or disease states might influence this accumulation.
Methods: In this study, we applied scWGS to neurons from the prefrontal cortex of CTE brains. Using PTA (Primary Template-directed Amplification), with LiRA (Linked-Read Analysis) and SCAN-SNV (Single Cell ANalysis of SNVs) computational analyses to distinguish sSNVs from amplification artifacts, we compared the rates of sSNV accumulation in CTE and control brains.
Results: We found a significant increase of hundreds of sSNVs in CTE as compared with age-matched controls. Additionally, we identified specific mutational signatures more abundant in CTE than in controls, distinct from the composition of mutational signatures in AD, providing insight into potential pathogenic mechanisms of CTE.
Conclusions: Since CTE is hypothesized to be caused by exposure to repetitive head trauma, its areas of pathological overlap with other neurodegenerative diseases, such as AD, make CTE a unique model for studying the effects of molecular pathways in neurodegeneration.

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Mapping neocortical thorn-shaped astrocytes in trauma, aging and neurodegeneration

K Bieniek[1], D Dickson[2]
[1]UT Health San Antonio; [2]Mayo Clinic Florida

Background: Aging-related tau astrogliopathy (ARTAG), namely thorn-shaped astrocytes (TSA) and granular/fuzzy astrocytes, is an increasingly recognized finding in the postmortem brain of elderly individuals. Additionally, its presence is notable, yet unclear, in the setting of antecedent repetitive head injuries and chronic traumatic encephalopathy pathology. This study's objective is to map and describe neocortical TSA across the lifespan, with and without a history of head trauma and/or neurodegenerative comorbidities.
Methods: Tissue sections from the superior/middle frontal gyri were immunohistochemically stained for phosphorylated tau (AT8) and screened in 778 brain autopsy cases (avg. age 65, range 7-100) from the Mayo Clinic Tissue Registry. Limited clinical data was derived from obituaries, yearbooks, and Rochester Epidemiology Project code sets. A subset of cases ($N = 315$) with additional temporal and parietal cortical tissue were further assessed to detail TSA distribution across the neocortex. ARTAG was classified in accordance with consensus criteria (ex: white matter, subpial, perivascular, etc.).
Results: Sixty-seven cases (8.6%) had frontal cortex TSA, including 42 subpial/perivascular-only, 17 white matter-only, and 8 mixed. ARTAG cases were significantly older than cases without (75.5 vs. 64.0 avg. age; $p < 0.0001$), and only 3% of cases ages 55 years and younger demonstrated ARTAG. Alzheimer's disease (AD) neuritic plaques were found in 23.5% of cases. Cases with frontal cortex white matter TSA were more likely to have comorbid AD (76%) than cases with subpial/perivascular TSA (36%; $P = 0.0014$). No differences were observed between TSA types and contact sports history. Of the cases with multiple cortices, TSA were observed in similar frequencies across cortices (13-15%) with 51 cases bearing TSA in a single region and 35 in multiple regions.
Conclusions: Neocortical ARTAG becomes more frequent with advanced age across the frontal, temporal and parietal lobes. Specific TSA patterns/morphologies are related to other comorbidities, and thus, mapping ARTAG is informative in understanding its role in neurodegeneration.

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TDP-43 Positive Inclusions in Non-Central Nervous System Tissue: A Potential Biomarker of TDP-43 Associated Disease

J Mingrino, M Punsoni
Brown University, Rhode Island Hospital

Background: Amyotrophic lateral sclerosis (ALS) is a progressive, fatal neurodegenerative disease with clinical heterogeneity and variable etiopathogenesis. ALS shares a neuropathologic substrate with Frontotemporal dementia (FTD), characterized by cytoplasmic Transactive response DNA-binding protein 43 (TDP-43) inclusions in the central nervous system (CNS). Several studies have suggested TDP-43 as an ideal biomarker candidate to assess premortem disease. We sought to further evaluate and describe TDP-43 inclusions in non-CNS tissue in TDP-43 Proteinopathy.
Methods: Between 2010-2021, we identified archival cases of clinical-neuropathologic ALS or ALS-FTD and reviewed clinical symptoms and pathology (CNS and non-CNS), including TDP-43 immunohistochemistry.
Results: We identified three patients (age range: 53-85y; 1M, 2F) with ALS and one who met criteria for FTD. The ALS patients exhibited slow (348 months) and intermediate (84 months) disease progression. In ALS-FTD, dementia preceded rapidly progressive (48 months) motor neuron disease. All showed motor cortex skein-like inclusions with variable anterior horn morphology. Slow disease progression was associated with constipation and volvulus, rare motor cortex skein-like inclusions, variable skeletal muscle inclusions, and granular gastrointestinal (GI) inclusions (myenteric plexus). Intermediate progression was reported with GI dysmotility and thick, granular GI inclusions (lamina propria and myenteric plexus). Rapid disease revealed GI-related symptoms with CNS, GI, and skeletal muscle inclusions, with dense-round GI aggregates (muscularis externa). Skeletal muscle showed denervation, atrophy, and variable inclusion morphology in cases with long disease duration.
Conclusions: Herein we show that TDP-43-associated disease may harbor variable inclusions in gastrointestinal and skeletal muscle tissue, supporting non-CNS tissue sampling. Evaluating distinctive TDP-43 morphology represents a mechanism for disease stratification. Our investigation suggests TDP-43 as a useful marker to detect and potentially monitor disease status and underscores the importance of detailed clinical GI symp-

tom assessment in all patients but particularly in patients with motor neuron disease, which may further inform colonoscopic tissue sampling.

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SCA34: A neuronal lysosomal lipid storage disorder sharing pathology features with neuronal ceroid lipofuscinosis and peroxisomal disorders

B Ellezam[1], J Michaud[2]
[1]Université de Montréal - Sainte-Justine Hospital; [2]University of Ottawa

Background: Spinocerebellar ataxia 34 (SCA34) is a late-onset progressive ataxia caused by a mutation in ELOVL4, a gene involved in the biosynthesis of very long-chain fatty acids (VLCFAs). Neuropathological data on SCA34 is limited to a recent report describing pontocerebellar atrophy, PAS-positive macrophages in the pontine base and widespread oligodendroglial pathology with vacuolar white matter degeneration in a Japanese patient with the ELOVL4 W246G mutation.
Methods: Post-mortem neuropathological examinations of four SCA34 patients with the ELOVL4 L168F mutation and comparison with age-matched controls.
Results: Gross examination revealed selective ponto-cerebellar atrophy. On light microscopy, pontine base showed neuronal loss and storage of an autofluorescent lipopigment positive on oil red O and PAS and negative on Alcian blue and LFB. Among the swollen neurons were abundant macrophages laden with a material with similar histochemical profile as in neurons except for the lack of autofluorescence and oil red O positivity and the presence of needle-like birefringent inclusions. In dentate nucleus neurons, atrophy was milder than in the pons and the coarser storage material was LFB-positive, closely resembling lipofuscin. On electron microscopy, dentate nucleus neurons showed neuronal storage of tridimensionally organized trilaminar spicules within otherwise normal lipofuscin, while in the more affected pontine base neurons lipofuscin was often completely replaced by the storage material. Storage macrophages were tightly packed with stacks of unorganized trilaminar spicules, reminiscent of the storage material seen in peroxisomal disorders and thought to represent VLCFAs incorporated in complex polar lipids.
Conclusions: In summary, we provide histochemical and ultrastructural evidence that SCA34 is a lipid storage disease, the first among the currently known SCAs, and that the storage lipid is accumulating within neuronal lipofuscin. Our findings suggest that the storage lipid is similar to the one accumulating in peroxisomal disorders and provide the first example of neuronal storage of this type in a neurodegenerative disease.

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Somatic genetic profiling of PRNP in Heidenhain variant and sporadic Creutzfeldt-Jakob Disease

G McDonough[1], K Morillo[2], R Doan[2], B Appleby[3], C Kim[3], M Cohen[3], J Safar[3], A Huang[2], C Walsh[2], M Miller[4]
[1]Brigham and Women's Hospital; [2]Boston Children's Hospital; [3]Case Western Reserve University; [4]Brigham and Women's Hospital, Boston Children's Hospital

Background: Creutzfeldt-Jakob Disease (CJD) is the most common human prion disease, associated with pathologic misfolding of the prion protein (PrP), which is encoded by the PRNP gene. Of human prion disease cases, ~1% are transmitted by exogenous misfolded PrP, ~15% are inherited, and ~85% are sporadic (sCJD). Familial prion disease is inherited through autosomal dominant gain-of-function germline mutations in PRNP, while the cause of sCJD is unknown. Emerging evidence in human brain aging and age-related diseases has shown abundant somatic mutations, many with functional significance.
Methods: To investigate the hypothesis that somatic mutations in PRNP may underlie sCJD, we performed ultra-deep sequencing of PRNP in a cohort of 205 sCJD cases and 160 age-matched non-disease controls. We included 5 cases of Heidenhain variant sporadic CJD (H-sCJD), where initial visual symptomatology and comparative neuropathology implicate possible somatic mutation in focal initiation of prion formation, and examined primary (Brodmann area 17) and secondary visual cortex (Brodmann areas 18 and 19), frontal cortex (Brodmann area 46), and cerebellum. We performed multiple independent primer PCR sequencing (MIPP-seq), using overlapping amplicons with average depth of ~8,000X across the PRNP coding region, and identified somatic variants using MosaicHunter. An allele mixing experiment showed positive detection of variants in bulk DNA at a variant allele fraction as low as 0.1%.
Results: Using stringent calling criteria, we identified somatic PRNP variants in 9 individuals in our cohort. We did not observe increased somatic variants in H-sCJD cases or overall sCJD, compared to controls, nor did we observe somatic variants for the known germline pathogenic alleles, P102L, D178N, or E200K.
Conclusions: In conclusion, ultra-deep PRNP sequencing did not identify pathogenic somatic mutations in H-sCJD or a broader cohort of sCJD, suggesting that somatic mutations in PRNP may not play a major role in sporadic prion disease.

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Association of repetitive head impacts and chronic traumatic encephalopathy with neurodegeneration in the cortical sulcus

R Nicks[1], A Shah[1], S Stathas[1], D Kirsch[1], S Horowitz[1], L Fishbein[1], N Saltiel[1], N Aytan[1], A Boden[1], S Calderazzo[1], M Butler[1], F Faheem[1], K Cormier[1], R Mathias[1], W Xia[1], G Meng[2], J Cherry[1], V Alvarez[1], B Huber[1], A McKee[1], T Stein[1]
[1]Boston University; [2]VA Bedford Healthcare System

Background: Atrophy and neuronal and synaptic loss are consistent features of neurodegenerative diseases. Chronic traumatic encephalopathy (CTE) is a neurodegenerative dis-

ease characterized by abnormal tau accumulation concentrated at the sulcal depths and caused by exposure to repetitive head impacts (RHI). However, regional cortical atrophy, synaptic, and neuronal loss have not been quantitatively assessed in CTE.

Methods: We measured cortical thickness and neuronal density (NeuN immunohistochemistry) within the gyrus and sulcus and synaptic density (α-synuclein, a pre-synaptic marker, and PSD-95, a post-synaptic marker, Meso Scale Discovery immunoassays) within the gyrus of the dorsolateral prefrontal cortex (DLPFC) in post-mortem contact sport athletes ($n = 185$) as well as non-athlete controls ($n = 52$).

Results: Cortical thickness and neuronal density were significantly reduced in both low and high-stage CTE compared to controls within the sulcus ($p's < 0.05$). Levels of α-synuclein were also reduced in low and high-stage CTE ($p's < 0.01$) compared to controls, but levels of PSD-95 showed greater variance in CTE. Multiple linear regression models showed that years of contact sports play (a proxy for RHI exposure) was associated with cortical thinning ($p < 0.001$) and with neuronal loss ($p = 0.041$) in the sulcus of the DLPFC, adjusting for age. When cortical tau pathology burden was included in the model, it completely accounted for neuronal loss, suggesting that RHI is associated with neuronal loss largely through the development of tau pathology. Conversely, the association between RHI exposure and cortical thinning was largely tau-independent, suggesting other factors, such as synaptic remodeling, might underlie cortical thinning in the sulcus.

Conclusions: Overall, we demonstrate an association between RHI exposure, neuronal loss, and cortical thinning in the DLPFC sulcus, which may inform new pathological and imaging features that aid in the diagnosis of CTE. Future studies should examine the sulcal predisposition and increased variability of synaptic loss in CTE.

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Huntington disease is associated with increased risk of concomitant TDP-43 encephalopathy

D Child[1], J Ariza Torres[2], S Jayadev[2], T Bird[2], C Keene[2], C Latimer[2]
[1]Department of Laboratory Medicine and Pathology, University of Washington; [2]University of Washington

Background: Huntington disease (HD) is a genetic neurodegenerative disease caused by a CAG repeat expansion in the HTT gene. Despite a single genetic etiology, the resulting phenotype is heterogenous, and clinical presentations can vary even within the same family. Several recent reports have described amyotrophic lateral sclerosis (ALS) occurring in HD patients, and our brain bank has received donations from two unrelated individuals in the past 25 years who presented with motor neuron disease. On neuropathologic evaluation, both individuals had findings characteristic of both HD and FTLD-ALS. Given the extremely low probability of these rare diseases co-occurring by chance alone, we hypothesize that mutant HTT increases risk of TDP-43 encephalopathy.

Methods: The prevalence of TDP-43 encephalopathy was determined in a cohort of forty HD brains from the UW brain repository. Five regions per brain were stained with a multiplex immunoassay to highlight HTT and TDP-43 inclusions, followed by digital quantitation and manual validation.

Results: TDP-43 inclusions were found in 30 out of 40 HD brains. The mean age among all positive cases trended older than negative cases (68 vs. 58.3 years, respectively). Of the 30 positive cases, eleven were older than 75 years; conversely, every negative case was 74 years or younger. Seven of the positive cases had an anatomic distribution and age consistent with limbic-predominant age-related TDP-43 encephalopathy (LATE), but most had a non-stereotypical distribution. TDP-43 and HTT inclusions only rarely colocalized within the same neuron, and there was no correlation between TDP-43 pathology and CAG repeat length.

Conclusions: These data suggest that HTT mutation is associated with increased risk of concomitant TDP-43 pathology. While the clinical implications and underlying cellular mechanisms are currently being elucidated, the increased prevalence of TDP-43 pathology is an intriguing possible contributor to phenotypic heterogeneity in HD.

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Tau Pathology-Associated Synaptic Changes in Primary Age-Related Tauopathy

M Morris, G Coste, J Redding-Ochoa, H Guo, A Graves, J Troncoso, R Huganir
Johns Hopkins University School of Medicine

Background: Primary age-related tauopathy is characterized by the age-associated aggregation of tau in the brain. High pathologic tau stage (Braak stage) or a high burden of hippocampal tau pathology are associated with cognitive impairment in PART. However, the underlying mechanisms are not well understood. In many neurodegenerative diseases cognitive impairment correlates with synaptic loss, raising the question of whether there are synaptic changes in PART.

Methods: To investigate synaptic changes in PART, we compared synaptic immunolabeling in cases of definite PART with controls and AD cases in the hippocampus. The tissue was immunolabeled for the pre-synaptic protein synaptophysin, which is widely used as a surrogate to measure synaptic loss in human tissue, and for phospho-tau (pS202/T205, AT8 antibody). As cognitive impairment in PART has been associated with both the burden of hippocampal tau pathology and Braak stage, we compared both measures to changes in the synaptophysin signal in multiple discrete regions of the hippocampus.

Results: Loss of synaptophysin puncta and intensity was associated with either a high burden of neuritic tau pathology or Braak stage IV disease in the CA2 region in the hippocampus in PART. A smaller effect on synaptophysin intensity was present in CA3 in PART associated with a high burden of neuritic tau pathology or Braak IV disease. Loss of synaptophysin was

also observed in AD, but the overall pattern of loss was distinct from that seen in PART.

Conclusions: These novel findings provide evidence for synaptic alterations associated with high tau pathology in PART, and are suggestive of synaptic loss in PART in the CA2 region of the hippocampus. It also suggests that the pattern of synaptic changes in PART may be distinct from those seen in Alzheimer's disease.

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Anterior insula TDP-43 pathology in LATE and other neurodegenerative diseases

R Lochner[1], A Arumanayagam[2], S Powell[2], J Masdeu[2], B Pascual[2], S Appel[2], M Cykowski[2]
[1]Department of Pathology and Genomic Medicine, Houston Methodist Hospital; [2]Houston Methodist Hospital

Background: Limbic-predominant age-related TDP-43 encephalopathy (LATE) is a common pathology in dementia, often co-occurring with Alzheimer's (AD) and Lewy body disease (LBD) pathologies. LATE neuropathologic change (LATE-NC) begins within the amygdala region (Stage 1), but the progression of TDP-43 pathology to other regions requires further investigation. We hypothesized that anterior insula would be one important focus in the progression of LATE-NC, based on its anatomic proximity, connectivity, and functional similarities to amygdala region.

Methods: Phospho-TDP (pTDP) pathology was assessed in 100 autopsied patients (median age 77 years, 54 men, 46 women) with intermediate and high AD neuropathologic change, LBD, and/or LATE, as well as other TDP-43 proteinopathies (ALS, late onset FTLD-TDP) and primary tauopathies. Within each subject, anterior and posterior insular cortices were examined at the levels of nucleus accumbens and thalamus, respectively. Pathologic assessments were made blinded to clinical and pathologic data.

Results: Insular cortex pTDP pathology was present in 31% of study patients, most commonly in the form of neuronal inclusions and short neurites in lamina II, and less commonly as subpial processes. pTDP pathology was limited to anterior insula (39% of positive cases), or occurred in both anterior and posterior insula (61%) - no examples had pTDP in posterior insular cortex only. Likewise, inclusion density was significantly greater in anterior insula across all conditions ($p < 0.001$). Among patients with LATE-NC, insula pTDP pathology was seen in 38%, occurring in Stage 1 (8%), Stage 2 (38%), and Stage 3 (85%) LATE-NC.

Conclusions: Anterior (agranular) insular cortex is involved by pTDP lesions in LATE-NC, and other conditions (e.g., ALS), and precedes involvement of posterior (granular) insula. In LATE-NC , involvement of anterior insula often occurred in tandem with striatal and basal forebrain pTDP pathology, together possibly representing an important step in the progression of LATE-NC outside of medial temporal lobe.

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An autopsy series of seven genetically confirmed cases of chorea acanthocytosis (VPS13A disease)

R Ditzel[1], R Walker[2], M Nirenberg[2], A Tetlow[3], K Lind-Watson[2], K Farrell[2], E Thorn[2], D Dangoor[2], R Gordon[2], C De Sanctis[2], G Miltenberger-Militenyi[4], J Humphrey[2], J Crary[2]
[1]Icahn School of Medicine at Mount Sinai, Departments ofPathology, Artificial Intelligence and human health, Neuroscience, Friedman Brain Institute, Ronald M. Loeb Center for Alzheimer's Disease, and Neuropathology Brain Bank & Research CoRE; [2]Icahn School of Medicine at Mount Sinai; [3]New York University; [4]University of Lisbon

Background: Vacuolar protein sorting 13 homolog A (VPS13A) disease, historically known as chorea-acanthocytosis, is a rare neurodegenerative disorder caused by biallelic mutations in VPS13A, resulting in reduced or absent protein. Neuropathological features include neuronal loss in the striatum, most prominently in the caudate nucleus, with associated marked astrogliosis. There are no other known disease-specific cellular changes (e.g., protein aggregation). VPS13A localizes to contact sites between subcellular organelles, consistent with its recently-identified role in lipid transfer between membranes. To date, autopsy reports have been limited, often lacking genetic or biochemical diagnostic confirmation.

Methods: In this study, brain tissues, clinical data, and other diagnostic data from seven clinically typical cases of VPS13A disease were collected from contributing centers. Tissues underwent routine, special, and immunohistochemical staining focused on neurodegeneration. Electron microscopy was performed in one case.

Results: Immunoblots confirmed the loss of VPS13A protein in some cases, and sequencing identified VPS13A mutations in all cases, including novel variants. Gross examination showed severe striatal atrophy. Microscopically, there was neuronal loss and astrogliosis in affected regions. Luxol Fast Blue staining showed variable lipid accumulation with diverse morphologies, a novel finding that was further demonstrated by electron microscopy. Two cases had comorbid Alzheimer's neuropathologic changes; one had brainstem-predominant Lewy body pathology in the absence of clinical parkinsonism. We also noted the variable presence of rare degenerating p62- and ubiquitin-positive cells in affected regions. Calcifications were present in three cases, being extensive in one

Conclusions: We present the largest autopsy series of biochemically- and genetically-confirmed VPS13A disease and report a detailed survey of histopathological findings.

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P-tau Pathology in the Medial Temporal Lobe in Chronic Traumatic Encephalopathy

G Kureli[1], D Kirsch[2], B Abdolmohammadi[2], T Stein[2], B Huber[2], V Alvarez[2], J Mez[2], M Alosco[2], A McKee[2]

[1]Boston University, Neurophotonics Center; [2]Boston University, School of Medicine

Background: Chronic traumatic encephalopathy (CTE) is a neurodegenerative tauopathy caused by exposure to repetitive head impacts. CTE is characterized by neuronal phosphorylated tau (p-tau) aggregates, that are initially focal, perivascular, and restricted to the cortex in mild disease (Low CTE, McKee stage I and II). As the disease progresses, p-tau pathology becomes widespread with severe involvement of the medial temporal lobe (MTL), diencephalon, and brainstem (High CTE, McKee stage III-IV).
Methods: We evaluated a postmortem cohort of 390 brain donors with CTE without comorbid neurodegenerative disease, using linear regression to analyze the associations between MTL p-tau pathology (i.e., semiquantitative neurofibrillary tangles (NFTs) in CA1,CA2, CA4, hippocampal neuronal loss, entorhinal NFTs, and amygdala NFTs) and CTE disease severity (i.e., McKee stages I-IV), and informant-reported cognitive and daily function as assessed by the Cognitive Difficulties Scale (CDS) and Functional Activities Questionnaire (FAQ), respectively. Analyses were controlled for age.
Results: Of the 390 brain donors, the mean (SD) age was 52.67 (18.06), 90 were Black, and 348 were American football players. 212 (54.4%) had Low CTE (stages I and II) and 178 (45.6%) had High CTE (stages III and IV). NFTs first appeared in the entorhinal cortex in stage II CTE, with progressive increases with stage and age ($p < 0.001$). NFTs next appear in the amygdala and hippocampus in stage III CTE, with progressive increases with stage and age ($p < 0.001$). Hippocampal neuronal loss was significantly associated with CDS and FAQ scores ($p = 0.006$ and < 0.001, respectively). Additionally, CA2 NFTs were significantly associated with CDS ($p = 0.024$).
Conclusions: These observations highlight the differential involvement of the MTL structures in CTE progression and suggest that CA2 NFTs and hippocampal neuronal loss contribute to cognitive symptoms.

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Chronic Traumatic Encephalopathy and Glioblastoma: Concurrence or Predisposition?

R Alfattal, B Shine, M Felicella
University of Texas Medical Branch - Galveston

Background: Chronic traumatic encephalopathy (CTE) is a neurodegenerative disorder associated with repetitive head trauma. Gliomas arising in the setting of traumatic brain injuries (TBI) have been reported extensively in the literature, including many theories regarding gliomagenesis. However, there are few reports of gliomas arising in the context of CTE.
Methods: We report a case of a 78-year-old man with a 13-year history of slowly progressive dementia.
Results: The patient was diagnosed clinically with Alzheimer's disease. In the last few months of his life, he developed sudden rapid decline with worsening memory impairment, aphasia, depression, paranoia, and physical aggression. He was a semi-professional football player with a history of multiple concussions in his youth and early adulthood. At autopsy, his brain showed mild cortical atrophy, significant atrophy of his mammillary bodies, ventriculomegaly, cavum septum pellucidum, and a necrotic mass in his right parietooccipital lobe. Microscopically, there was no evidence of Alzheimer disease neuropathologic change or Lewy body pathology. However, there were multiple perivascular aggregates of phosphorylated tau-containing neurons and astrocytes at the depths of cortical sulci (i.e., the pathognomonic CTE lesion). Staging with the second NINDS/NIBIB consensus criteria revealed "High CTE" with a score of 10/10 points as well as changes of age-related tau astrogliopathy (ARTAG). An IDH-wildtype glioblastoma was also present in addition to neurodegenerative changes.
Conclusions: Although proposed causal relationships between TBI and glioma are debated in the literature, little is known about possible associations between CTE and glioma development. Hypotheses include recruitment of progenitor cells in response to inflammation and mutagenic effects of oxidative stress. In addition, studies have found evidence of DNA damage, DNA-repair defects, and cellular senescence in brains of athletes with repeated concussive and subconcussive TBI. This substrate may potentially predispose these patients to development of glial neoplasms.

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Neuropathologic findings of mixed Kufs syndrome with homozygous CTSF K331Nfs*14 mutation

N Ghayal[1], S Roemer[1], P Tipton[1], E Selner[2], D Peck[2], P Jiang[1], D Oglesbee[2], N Graff-Radford[1], D Dickson[1]
[1]Mayo Clinic Florida; [2]Mayo Clinic Rochester

Background: The identification of causative genes in neuronal ceroid lipofuscinosis (NCL) has greatly improved detection in children. However, the detection of adult or adolescent-onset NCL (ANCL) remains challenging due to the delayed manifestation and varying constellations of clinical symptoms. ANCL often lacks the retinal involvement more commonly observed in childhood NCL and traditionally presents as either progressive myoclonic epilepsy, known as Kufs disease type A (KD-A), or dementia with motor disturbance, known as Kufs disease type B (KD-B).
Methods: Here, we describe the neuropathologic findings of a woman of Indian ancestry who presented at age 27 with initial tonic-clonic seizures and would later develop facial hyperkinetic movements, severe cognitive impairment with behavioral and neuropsychiatric disturbance, cerebellar dysfunction, extrapyramidal signs, and eventual akinetic mutism during a 27-year disease course. She was negative on multiple EEGs, and T2-FLAIR studies later in life demonstrated generalized diffuse cortical atrophy, corpus callosum atrophy, and mild vermian atrophy, as well as ventriculomegaly. Her family history is

significant for a maternal grandmother with late dementia and a distant paternal relative with schizophrenia.
Results: Macroscopic findings at autopsy matched the neuroimaging findings. On histologic examination, all lobes contained swollen autofluorescent and diastase-resistant pigment-containing neurons predominantly in the pyramidal cortical layers and autofluorescent meganeurites in superficial cortical layers, consistent with neuronal ceroid lipofuscinosis. Immunohistochemically, there were PART-like/epilepsy-associated p-tau changes in the medial temporal lobe with a Thal phase 0. Ultrastructural examination revealed fingerprint-like structures and unusual granulolamellar complexes.
Conclusions: Postmortem next-generation sequencing revealed a novel homozygous indel mutation in CTSF (p.K331Nfs*14), thereby classifying the disease as NCL type 13 or CLN13.

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Association of TDP-43 proteinopathy with cognitive decline and Alzheimer's disease neuropathologic changes in the aging population

K Chang[1], J Redding[1], Y An[2], T Pylyukh[1], A Barrett[1], A Moghekar[1], S Resnick[2], J Troncoso[1]
[1]Johns Hopkins University School of Medicine; [2]National Institutes of Health

Background: TDP-43 neuropathologic changes are associated with Alzheimer's disease (AD) and cognitive impairment in advanced age population. However, the association of TDP-43 proteinopathy with AD neuropathologic changes (AD-NC) and its impact on different cognitive domains in the aging population are not fully understood.
Methods: We assessed TDP-43 neuropathologic changes and AD-NC in autopsied participants from the Baltimore Longitudinal Study of Aging (BLSA). All participants had normal cognition at enrollment and received regular longitudinal cognitive assessments. We used logistic regression analysis to model the relationship between CERAD neuritic plaque (NP) scores, Braak neurofibrillary (NF) stages and probability of TDP-43 proteinopathy. Linear mixed effects models were used to estimate the effect of TDP-43+ neuronal cytoplasmic inclusions (NCIs) on the longitudinal cognitive trajectories.
Results: 309 participants were included in the study. The average age at death was 88.5 years. 38.2% participants had TDP-43+ NCIs and neurites accompanied by nuclear clearance of TDP-43. Notably, among brains free of TDP-43+ NCIs, 23% had nuclear clearance of TDP-43. Age at death, female sex, high NP score, and high NF stage significantly increased the odds of TDP-43+ NCIs presence. TDP-43+ NCIs positivity showed significant association with declines in the cognitive trajectories of California Verbal Learning Test (CVLT) immediate recall, Card Rotation Test, MMSE and category fluency test. The associations of TDP-43+ NCIs positivity with declines in CVLT immediate recall (memory function) and Card Rotation Test (visual-spatial function) remained significant after adjusting for AD-NC.
Conclusions: 1. TDP-43 proteinopathy is associated with AD-independent declines in memory and visuo-spatial ability in the aging population. 2. Nuclear clearance of TDP-43 precedes the accumulation of this protein in NCIs and neurites, suggesting that it is an early event in TDP-43 proteinopathy, a situation that has been proposed as sufficient for causing loss of TDP-43 function in neurons.

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An Autopsy Case of Corticobasal Degeneration with A History of Acute Restlessness and Stupor

M Mizutani[1], Y Saitoh[2], T Sano[3], S Shimizu[3], N Satake[3], M Takao[3]
[1]Laboratory and Internal Medicine; [2]Tokyo Metropolitan Neurological Hospital; [3]National Center of Neurology and Psychiatry

Background: Some cases of corticobasal degeneration (CBD) show neuropsychiatric symptoms, including frontal lobar symptoms. However, case reports of stupor with CBD are rare. Here, we report an autopsy case of CBD with a history of acute restlessness and stupor.
Methods: A 71-year-old woman was transferred to our hospital from a psychiatric hospital for COVID-19 treatment. She had suddenly developed restlessness at age 63, and started to ask for help, bizarrely fearing an earthquake without any trigger. She developed abnormal behaviors such as repeatedly turning her cell phone on and off while saying she was typing a text message. Despite treatment with psychotropic drugs, she fell into a state of stupor and admitted to a psychiatric hospital. Following radiological examination, she was diagnosed with frontotemporal degeneration. When she was transferred to our hospital, she showed akinetic mutism. Despite treatment, she died of respiratory failure ten days after infection. We conducted an autopsy with consent from the bereaved family.
Results: The autopsy revealed that her brain weight was 885 g. The cerebrum and brainstem showed severe atrophy, and immunostaining by anti-RD4 antibody showed astrocytic plaques, pretangles, coiled body, and threads predominantly in the frontal lobe, basal ganglia, limbic system, and brainstem. pTDP43 was mildly accumulated in the basal ganglia and frontal lobe. We pathologically diagnosed her with CBD.
Conclusions: We experienced a rare autopsy case of CBD with acute restlessness and stupor. Considering the median life expectancy of CBD (7 to 8 years), and the frontal cortex-predominant pattern of tau accumulation, her rare psychiatric symptoms might be interpreted as prodromal symptoms of her CBD. Further autopsy research is warranted for better understanding of psychiatric symptoms triggered by CBD.

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Isodendritic Core Pathology in Chronic Traumatic Encephalopathy Increases with Disease Stage and Years of Play

D Kirsch, G Kureli, B Abdolmohammadi, T Stein, B Huber, V Alvarez, J Mez, M Alosco, A McKee
Boston University School of Medicine

Background: The isodendritic core (IC) is composed of brainstem and basal forebrain nuclei that project widely to the allo- and neocortex. The physiological and behavioral roles of the IC are diverse; the substantia nigra provides input to the basal ganglia that modulates movement, the median raphe nuclei influences sleep/wake cycles, the locus coeruleus modulates arousal and attention, and the substantia inominata is involved in activation and cognition. Early involvement of IC nuclei has been described in Alzheimer's disease, Parkinson's disease, and frontotemporal dementia, and is believed to contribute to non-cardinal symptoms. Chronic traumatic encephalopathy (CTE) is a neurodegenerative tauopathy linked to exposure to repetitive head impacts (RHI).
Methods: In a postmortem cohort ($n = 392$) of brain donors with CTE without comorbid neurodegenerative disease, we used linear regression modelling to analyze the associations between IC nuclei pathology (semiquantitative neurofibrillary tangles (NFTs), neurites, and neuronal loss scores) and CTE disease severity, RHI exposure duration (years of contact sport play), and informant-reported cognitive and daily function as assessed by the Cognitive Difficulties Scale (CDS) and Functional Activities Questionnaire (FAQ), respectively. Analyses controlled for age.
Results: Overall, isodendritic NFT scores increase with disease stage, with the earliest increases in the locus coeruleus and the latest in the median raphe nuclei. Neuronal loss occurred at later disease stages than NFT accumulation. Increased RHI exposure was directly associated with p-tau pathology for all IC regions ($ß=0.576$, $p < 0.001$). NFTs and neuronal loss in the substantial nigra were associated with increased CDS scores ($ß=0.171$, $p = 0.005$) (i.e., worse cognitive function), and neuronal loss in the substantia nigra and locus coeruleus were associated with increased FAQ scores($ß=0.185$, $p < 0.001$) (i.e., worse daily function).
Conclusions: These results demonstrate the vulnerability of the isodendritic core nuclei to p-tau pathology and neuronal loss in CTE, and suggest that their involvement contributes to cognitive and functional symptoms.

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Isolated Chronic Traumatic Encephalopathy-like Pathology Adjacent to Infiltrative and Non-infiltrative White Matter Lesions

M Shields[1], M Scanlon[1], D Perl[1], D Priemer[2]
[1]Uniformed Services University School of Medicine, Bethesda, MD, USA; [2]The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

Background: The pathognomonic lesion of chronic traumatic encephalopathy (CTE) is defined by neuronal accumulation of phosphorylated-tau in a perivascular distribution at a cortical sulcal depth. CTE has been mainly associated with repetitive impact-type traumatic brain injury (TBI) from contact sports, but also from impact-type TBI unrelated to sports (e.g., domestic abuse, epilepsy, head-banging behaviors). Rarely, CTE pathology has corresponded with single moderate-to-severe TBIs, including in relation to lobotomy sites in formerly institutionalized psychiatric patients. Indeed, there are suggestions that underlying axonal injury may predispose to the characteristic tau accumulation of CTE. We present three cases wherein a CTE pathognomonic lesion was found adjacent to underlying white matter pathology.
Methods: Case One is a 41-year-old male with history of hereditary hemorrhagic telangiectasia (Osler-Weber-Rendu syndrome) and an arteriovenous malformation of the brain status-post resection, who died from pulmonary thromboembolism. Case Two is a 46-year-old male who died of glioblastoma. Case Three is a 52-year-old male with history of remote cerebral infarction who died of myocardial infarction.
Results: Isolated CTE lesions were found immediately adjacent to the underlying white matter pathology in all cases. Additional, more distant CTE lesions were not identified.
Conclusions: While cortical neurofibrillary changes adjacent to arteriovenous malformations have been reported, these have not been described with the specific features of CTE. To our knowledge, CTE pathology has also not been described with an underlying diffuse glioma or cerebral infarct. These cases may provide further insight regarding the nature of the CTE pathognomonic lesion, and present further evidence that CTE-like pathology may occur outside the context of repeated TBI. The information/content and/or conclusions herein do not necessarily represent the official position or policy of, nor should any official endorsement be inferred on the part of, USU, the DoD, the U.S. Government, or the Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

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CRISPR-based screens uncover novel molecular chaperones that modify polyglutamine protein aggregation in specific cellular compartments

B Ramani, M Kampmann
University of California San Francisco

Background: Polyglutamine (polyQ) diseases, including Huntington's disease, are caused by abnormally expanded polyQ tracts encoded in specific proteins. A strong body of evidence supports a pathogenic role of mutant protein misfolding and aggregation. Molecular chaperones, which assist proper protein folding, have importantly been shown to suppress polyQ protein aggregation. However, the endogenous chaperones that handle mutant polyQ proteins have not been defined in human cells. Moreover, the chaperones that operate in the nucleus, the main site of polyQ disease protein aggregation, are not known.

Methods: We developed novel fluorescent cellular models of polyQ-expanded protein aggregation localized to either the nucleus or cytoplasm, and with the aggregates detectable by fluorescence resonance energy transfer. Using HEK293T cells and fluorescence-activated cell sorting, we conducted deep, unbiased CRISPR interference screens examining all known endogenous chaperones and co-chaperones to identify modifier hits, defined as gene knockdowns significantly accelerating aggregation of the fluorescent reporter.
Results: The CRISPR interference screens confirmed several top hits (HSPA8, DNAJB6, and DNAJB1) previously known to suppress polyQ protein aggregation, while additionally verifying their efficacy in both cellular compartments. Screens examining both compartments additionally revealed several strong hits previously unexplored in polyQ diseases, including multiple co-chaperones and tetratricopetide repeat domain-containing proteins. Moreover, the screens uncovered chaperones preferentially affecting nuclear or cytoplasmic polyQ protein aggregation, supporting possible compartment-specific differences of the endogenous chaperone network.
Conclusions: This study provides the first comprehensive view of the functional human endogenous chaperone network combating disease-associated protein aggregation, including in different cellular compartments. It also identifies several previously unexplored chaperones that could prove to be key regulators of polyQ protein aggregation and associated toxicity in the brain. Efforts to similarly define the chaperones that combat polyQ protein aggregation in neurons using a novel in vivo CRISPR screening platform are ongoing.

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Comparative single cell analysis in postmortem brains of patients with frontotemporal lobar degeneration with tau deposition

G Hargus[1], O Al Dalahmah[1], F Paryani[1], K Jakubiak[1], M DeTure[2], D Dickson[2], P Canoll[1], J Goldman[3], V Menon[1]
[1]Columbia University; [2]Mayo Clinic Jacksonville; [3]Columbia Universiy

Background: Frontotemporal dementia (FTD) is a heterogenous group of early-onset dementias that can be caused by deposition of hyperphosphorylated tau in neurons and glial cells in various regions throughout the central nervous system including the frontal and temporal lobes. Despite ongoing research, the mechanisms leading to neurodegeneration remain largely unknown.
Methods: Here, we performed single nucleus RNA sequencing (snRNA-seq) on the BA9 region of 12 FTD patients with mutations in the MAPT gene (P301L, N279K or 10 + 14), of 6 patients with progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD), and of 11 healthy control individuals. This analysis allowed us to identify gene expression profiles in neurons and glial cells in patient brains.
Results: We found altered gene signatures in glutamatergic and GABAergic neurons, in cortical neurons of different cortical layers, in microglia as well as in CD44+ and CD44- astrocytes. Despite the shared neuropathologic features between sporadic and genetically defined FTD, the cell-type specific gene expression changes were dependent on the genetic background of the donors. We also compared these gene expression profiles of FTD patients with those of patients with Alzheimer's disease carrying mutations in the presenilin 2 (PSEN2) gene identifying neuronal and glial signatures in primary versus secondary tauopathies.
Conclusions: Overall, this approach has the goal to characterize dysregulated pathways in neuronal and glial cells in FTD and to potentially identify therapeutic targets in this currently incurable group of neurodegenerative diseases.

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Characterization of Familial FTLD associated with MAPT mutation

M Bogdani[1], V Jadhav[2], N Liachko[1], C Keene[1], T Bird[1], C Latimer[1]
[1]University of Washington; [2]Univeristy of Washington

Background: Approximately half of patients with frontotemporal lobar degeneration exhibit aggregated and phosphorylated tau (FTLD-tau) at autopsy. Most FTLD-tau cases are sporadic, but a subset are caused by mutations in the gene encoding the protein tau, MAPT, providing a direct link between tau dysfunction and neurodegeneration. There are over 50 different known causal MAPT mutations and the pathology varies within and across different mutations. Further, the pathology of MAPT-associated FTLD-tau overlaps with the sporadic form. Given this heterogeneity and relative rarity of genetic FLTD-tau, it has been challenging to fully characterize the pathologic and genetic correlations and identify mechanisms of tau toxicity. Larger cohorts with extensive neuropathologic assessments are necessary to begin to bridge these gaps in knowledge.
Methods: Here we present a cohort of MAPT carriers including 18 individuals across 8 carrier families and representing 4 different mutations. Using standard immunohistochemistry we characterized tau distribution patterns, tissue integrity, neuroinflammation, and other pathologic proteins. This analysis was paired with molecular profiling using NanoString technology to assess for expression differences in 770 neurodegenerative disease related genes in which we compared MAPT mutation carriers to sporadic FTLD-tau and normal controls.
Results: Neuronal tau pathology was present in all cases, however the tau burden differed between brain regions and concomitant glial tau pathology was identified in only a subset of cases. Comorbid proteinopathy was also variable; 9 cases had amyloid plaques and 5 had TDP-43 pathology. NanoString analysis identified a small number of differentially expressed genes, implicating both unique and overlapping potential pathways underlying neurodegeneration in sporadic versus genetic FTLD.
Conclusions: This resource is ideal for addressing the question of neuropathological variation within and between MAPT mutation carrier families. The findings may ultimately help uncover key mechanisms of tau neurotoxicity and reveal potential therapeutic targets for tauopathies in general.

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The Mayo Clinic brain bank experience of archival cases submitted to the Pick International Consortium suggests a need for harmonized criteria

S Roemer, M Castanedes-Casey, N Perez, D Dickson
Mayo Clinic

Background: On behalf of the Pick International Consortium (PIC), we submit the Mayo Clinic experience on the diagnostic process of archival cases submitted from North America. Neuropathologically Pick's disease is diagnosed by the presence of round neuronal argyrophilic or tau-positive inclusions; Pick bodies (PB), these have been identified to consist of 3-repeat tau (3R).
Methods: Neuropathologists at the Mayo Clinic, Florida, were tasked by PIC to formulate a set of provisional diagnostic PIC-criteria and evaluate submitted cases. The rationale for the criteria was minimal work-up that reliably establishes the diagnosis in biopsies or autopsies. A Mayo-cohort consisting of 61 Pick autopsies served as an internal reference cohort since all these cases had been scored in 21 regions and undergone diagnostic work-up using Thioflavin-S, phosphorylated-tau, 3R-tau, 4R-tau, phosphorylated-TDP-43 and when relevant Gallyas silver-staining method, alpha-synuclein and p62. To fulfill PIC-criteria cases needed to demonstrate; presence of round inclusions, presence of 3R-positive round inclusions, absence of 4R-positive round inclusions, concomitant pathologies were acceptable. TDP-43 data was provided by individual centers and considered not stained if missing.
Results: Specimens were sampled from the hippocampus (89%) followed by the frontal cortex, temporal cortex or amygdala. 3% of submitted cases had TDP-43 staining, all negative consistent with the Mayo-cohort. 31% of cases failed PIC-criteria, these more frequently relied on non-hippocampal sections (24%) contrasting cases fulfilling PIC-criteria (non-hippocampal sections 5%). A failed diagnosis appeared driven by the presence of small round Alzheimer's-type tangles in cortical layer II or the end-plate, possibly mimicking round PBs, followed by the presence of both 3R and 4R positive Pick body-like inclusions, or by the presence of round limbic 4R-predominant inclusions.
Conclusions: These findings suggest the presence of argyrophilic or tau-positive round inclusions inadequately captures Pick's disease as a 3R-predominant tauopathy. A harmonized approach could increase the consistency in diagnosing this rare frontotemporal dementia.

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Mixed progressive supranuclear palsy (PSP) and multiple system atrophy (MSA): Report of four cases with heterogenous clinical presentations

D Dickson[1], M Armstrong[2], H Sekiya[1], R Uitti[1]
[1]Mayo Clinic; [2]University of Florida

Background: The brain bank for neurodegenerative disorders at Mayo Clinic Florida in Jacksonville has a focus on atypical parkinsonian disorders. The brain bank currently houses over 1,500 cases of Lewy body disease presenting with parkinsonism, dementia or both. It also houses 1,705 cases with pathologically-confirmed progressive supranuclear palsy (PSP) and 349 cases of pathologically-confirmed multiple system atrophy (MSA). In 2006 we reported the first case of mixed PSP and MSA in an 84-year-old woman who had a PSP syndrome with unexpected falls and vertical gaze palsy. To our knowledge there has been only one additional report of such a case (Takanashi M, et al. Acta Neuropathol 2002. PMID: 11837750).
Methods: Since then, three additional patients have been acquired by the Mayo Clinic brain bank. The Table summarizes differences between PSP, MSA, and mixed PSP-MSA cases. N Age Female FHx Braak stage Thal phase LBD VaD TDP-43 APOE4 MSA+PSP 4 79 50% 0 IV 3 0% 50% 0 50% MSA 349 86 48% 13% II 1 5% 13% 0 30% PSP 1705 75 45% 21% II 1 7% 24% 0 20% FHx = family history; LBD = Lewy body pathology; VaD = cerebrovascular pathology; APOE = apolipoprotein E4 genotype
Results: One was a 68-year-old man with parkinsonism, autonomic dysfunction and sleep disorder who had neuroimaging evidence of striatonigral degeneration (MSA-P); one was an 87-year-old man with parkinsonism and dementia without significant autonomic dysfunction or vertical gaze palsy (Parkinson disease dementia (PDD)), and one was a 77-year-old-woman with atypical parkinsonism and alien limb syndrome consistent with corticobasal degeneration (CBD).
Conclusions: Given the distinct clinical and pathological features, as well as different genetic risk factors for PSP (e.g., MAPT genetic variant) and MSA (no known genetic risk), we interpret the findings in these four patients to represent two independent disease processes within these individuals.

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Heterogeneous neuropathology in a pedigree with RAB39B-related Parkinson's disease

C Latimer, K Su, M Bogdani, D Chen, W Raskind, C Keene, T Bird, C Zabetian
University of Washington

Background: Parkinsonism refers to a clinical spectrum of symptoms including bradykinesia, rigidity, and resting tremor. When it is part of a neurodegenerative disorder the pathology is most commonly a synucleinopathy, but it can be associated with other proteins, including tau. In the small subset of patients with monogenic neurodegenerative parkinsonism, the pathologic findings are typically consistent within a family with respect to the proteinopathy. Here we present a family with monogenic parkinsonism and pathologic heterogeneity.
Methods: We previously identified a missense variant in the X-linked RAB39B gene (c.574G > A; p.G192R) in all seven affected individuals (5 males and 2 females) in a family who manifested a classic Parkinson's disease (PD) phenotype. Herein we describe the postmortem neuropathologic assessment of two affected males. Immunohistochemical staining of

the brain included α-synuclein, phospho-tau, phospho-TDP-43, amyloid ß and RAB39B.
Results: The younger brother (age 54) had unilateral symptom onset at age 31 and the older brother (age 65) was diagnosed with PD in his early 50s. Both brothers later developed severe dyskinesias and psychiatric symptoms, including psychosis, that were levodopa responsive, At autopsy both cases showed marked pallor of the substantia nigra with severe neuron loss and associated gliosis. The younger brother had Lewy body pathology in a neocortical distribution, but also an unusual pattern and burden of tau pathology that included neocortical neurofibrillary tangles, pretangles, and neurites in the absence of amyloid pathology. The older brother's brain, while showing a similar pattern of tau pathology, was notable for a lack of α-synuclein pathology. Accumulations of RAB39B were found in the neurons in the substantia nigra in the younger brother, but not in the older one.
Conclusions: These cases suggest that the molecular processes underlying RAB39B specifically, and monogenic parkinsonism in general, are complex and highlight the need for further clinical-genetic-pathologic correlations.

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Alpha-synuclein oligomers in Parkinson's disease with LRRK2 mutations

H Sekiya[1], Y Hashimoto[2], M Takata[2], S Koga[1], K Hasegawa[3], H Kowa[2], T Toda[4], Z Wszolek[1], D Dickson[1]
[1]Mayo Clinic; [2]Kobe University; [3]Sagamihara Hospital; [4]The University of Tokyo

Background: Mutations in leucine-rich repeat kinase 2 (LRRK2) are a frequent cause of familial Parkinson's disease (PD). Lewy-related pathologies (Lewy bodies and Lewy neurites) are late-stage aggregates of alpha-synuclein (aSYN) and are considered a hallmark of PD. Although clinical presentations of LRRK2-PD patients are similar to sporadic PD, some lack Lewy-related pathologies. The toxicity of αSYN oligomers, early aggregates of αSYN, has garnered attention, but their distribution in LRRK2-PD is unknown. We aimed to examine the distribution of αSYN oligomers in LRRK2-PD brains.
Methods: Brain samples from four LRRK2-PD patients with G2019S mutation and five with I2020T mutation were analyzed. Two G2019S and three I2020T samples lacked Lewy-related pathologies. Five control brains were also analyzed. αSYN oligomers were visualized by homotypic proximity ligation assay and examined in the brainstem, basal ganglia, limbic, and neocortex.
Results: All LRRK2-PD patients, including those without Lewy-related pathologies, had an accumulation of αSYN oligomers in all regions. Control brains showed little αSYN-PLA signals. The αSYN oligomer burden in the neocortex, brainstem, and hippocampus was significantly higher in LRRK2-PD patients with G2019S mutation than in those with I2020T mutation. In the entorhinal cortex, the αSYN oligomer burden was higher in LRRK2-PD patients without Lewy-related pathologies than in those with Lewy-related pathologies.
Conclusions: Our findings indicate widespread accumulation of αSYN oligomers in LRRK2-PD patient brains, suggesting that they may contribute to the pathogenesis of the disease, regardless of the presence of Lewy-related pathologies.

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Parkinson's disease with olfactory hypoplasia, a candidate for body-first propagation of Lewy body-related α-synucleinopathy

T Matsubara[1], S Murayama[1], T Tsukamoto[2], T Sano[2], M Mizutani[2], Z Tanei[2], Y Takahashi[2], M Takao[2], Y Saito[1]
[1]Tokyo Metropolitan Institute for Geriatrics and Gerontology, Tokyo, Japan; [2]National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan

Background: The propagation of Lewy body-related α-synucleinopathy is assumed to spread via two independent pathways: the olfactory bulb to amygdala pathway and the peripheral nervous systems to brainstem ascending pathway. This led to our proposal of "brain-first" versus "body-first" hypothesis of Lewy body-related α-synucleinopathy propagation. We here presented an autopsy case of Parkinson's disease with severe olfactory hypoplasia who apparently lacked the pathway starting from the olfactory bulb.
Methods: A full autopsy was done with comprehensive neuropathological studies of the central and peripheral nervous systems.
Results: The patient with a history of anosmia since childhood developed Parkinson's disease at the age of 56 years and died after 26 years of clinical course. Autopsy was performed 23 hours after death. The brain weighed 1402 g before fixation. Lewy bodies and Lewy neurites immunoreactive for anti-phosphorylated α-synuclein antibodies were widely distributed throughout the peripheral nervous systems, including the esophagus, heart, sympathetic ganglia, adrenal gland, and skin, as well as the brainstem, including the dorsal motor nucleus of the vagus nerve, locus coeruleus, substantia nigra, and Edinger–Westphal nucleus. In contrast, the limbic systems, including the amygdala, hippocampus, and transentorhinal and cingulate cortices, and the neocortex had relatively small amounts of inclusions. Other age-related changes were minimal (Braak NFT stage: I, Saito argyrophilic grain stage: I, Amyloid β: None, Phosphorylated TDP43: None).
Conclusions: As the malformation minimized the contribution of the olfactory bulb to amygdala pathway, this can be a

possible model case of Lewy body-related α-synucleinopathy accumulation derived from the peripheral nervous systems to brainstem ascending pathway: "body-first Lewy body disease."

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Pathoanatomical mapping of activated EIF2α in vulnerable brain regions in progressive supranuclear palsy

D Dangoor[1], K Whitney[2], M Krassner[3], K Farrell[2], H Ressler[3], T Christie[3], A Sharma[3], W Song[3], B Zhang[3], A Pereira[3], J Crary[3]
[1]Departments of Pathology, Neuroscience, Artificial Intelligence and Human Health, Friedman Brain Institute, Ronald M. Loeb Center for Alzheimer's Disease; [2]Icahn School of Medicine at Mount Sinai; [3]Icahn School of Medicine

Progressive supranuclear palsy (PSP) is a rare neurodegenerative movement disorder characterized neuropathologically by abnormal hyperphosphorylated tau (p-tau) aggregates in neurons, oligodendrocytes, and astrocytes, most notably in the peri-Rolandic cortex, basal ganglia, and brainstem. Most PSP cases are sporadic and linked to common structural genomic variation in the 17q21.31 MAPT locus. Genome-wide association studies have identified additional risk loci, including eukaryotic translation initiation factor 2 alpha kinase 3 (EIF2AK3). EIF2AK3 encodes a component of the unfolded protein response (UPR), a mechanism that restores protein homeostasis during endoplasmic reticulum (ER) stress. Studies have implicated dysregulation of UPR activation in multiple neurodegenerative diseases, including PSP. Our single-cell transcriptomic data suggests that EIF2 signaling is altered in PSP. To further investigate the role of the UPR in this disease, we performed a pathoanatomical study of the most selectively vulnerable brain regions and cell types in PSP. We assessed formalin-fixed paraffin-embedded postmortem brain tissue from PSP patients and controls using routine histological stains and immunohistochemistry to detect p-tau and the activated UPR marker phospho-EIF2α (pEIF2α). Sections were scanned, and whole-slide images were scored semi-quantitatively for neurodegeneration, p-tau burden (neuronal and glial), and pEIF2α burden. We found pEIF2α immunopositive cells in brain regions vulnerable to PSP and at higher levels than in protected regions. Levels of pEIF2α positively correlated with p-tau burden. These results support the hypothesis that dysfunction of the UPR may play a role in abnormal tau deposition and neurodegeneration in PSP. Exploring the upstream and downstream components of the UPR in response to tauopathy may provide further insight into the interplay between UPR activation and PSP pathology and progression.

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Neuropathological alterations in subjects initially diagnosed by polysomnography with isolated REM sleep behavior disorder

I Aldecoa[1], G Maya[2], N Vidal-Robau[3], C Gaig[2], J Santamaria[2], M Serradell[2], L Molina-Porcel[4], E Gelpi[5], Iranzo[2]
[1]Pathology Department and Neurological Tissue Bank of the Biobank, Hospital Clinic de Barcelona-CDB-IDIBAPS, Barcelona, Spain; [2]Sleep Center, Neurology Service, Hospital Clinic de Barcelona, Barcelona, Spain; [3]Pathology Department, Hospital Clinic de Barcelona-CDB-IDIBAPS, Barcelona, Spain; [4]Alzheimer's Disease and Other Cognitive Disorders Unit, Neurology Department, Hospital Clinic de Barcelona, Barcelona, Spain; [5]Division of Neuropathology and Neurochemistry, Department of Neurology, Medical University of Vienna, Vienna, Austria

Background: Isolated REM sleep behavior disorder (IRBD) is a parasomnia characterized by nightmares and dream-enacting behaviors emerging in REM sleep. More than 90% of IRBD patients at 15 years of diagnosis are at risk of developing Parkinson's disease (PD), dementia with Lewy bodies (DLB) and to a lesser extent multiple system atrophy (MSA). Polysomnography (PSG) is usually a required technique for adequate diagnosis, but PSG confirmed IRBD is a fraction of all IRBD population.
Methods: Eighteen patients with PSG confirmed IRBD were followed up until death and brain donation (2005-2020). Standard neuropathological evaluation and semiquantitative assessment of pathological alpha-synuclein (AS) deposits and the degree of gliosis and neuronal loss in multiple brain areas were performed.
Results: 94% of participants were male, with a mean age (+/- SD) of 71 ± 6 years at IRBD diagnostic PSG and of 80 ± 6 years at death. Clinical antemortem diagnosis was DLB (n = 10), PD (n = 5) and IRBD (n = 3); 17 patients had Lewy body disease (LBD) and one patient had MSA. All had frequent brainstem alpha-synuclein pathology and 94% had moderate neuronal loss in the locus coeruleus/subcoeruleus complex. A caudo-rostral gradient of alpha-synuclein pathology from midbrain over limbic and neocortical areas was observed in LBD-IRBD patients that progressed to PD or DLB. All patients had some degree of co-pathology: 12 had Alzheimer's disease neuropathological change (ADNC), 11 age-related tau astrogliopathy, 4 argyrophilic grain disease, 3 progressive supranuclear palsy and 3 limbic predominant age-related TDP-43 encephalopathy. The six patients with intermediate/high ADNC had neocortical LBD.
Conclusions: IRBD results mainly from an underlying LBD with prominent brainstem involvement and infrequently due to MSA. LBD neuroanatomical distribution is more in line

with a "body-first" progression hypothesis. Among age-related co-pathologies, ADNC is particularly frequent in the neocortical LBD stages and likely modulates the clinical phenotype, especially dementia.

FRIDAY POSTERS: Ophthalmic Pathology

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Protein aggregates associated with neurodegeneration in the retina and lateral geniculate nucleus

K Scherpelz, C Lee, D Keene
University of Washington

Background: Histopathologic changes of neurodegenerative disease in the visual system including neuroretina and its primary relay site the lateral geniculate nucleus (LGN) are not as well characterized as in the brain.
Methods: Ten brain-and-globe donors aged 58-90 underwent neuropathologic evaluation. Diagnoses from brain tissue included Alzheimer disease neuropathologic change (AD-NC, N = 7), tauopathies (frontotemporal dementia-tau [FTLD-tau, N = 1], age-related tau astrogliopathy [ARTAG, N = 5], argyrophilic grain disease [AGD, N = 3], chronic traumatic encephalopathy [CTE, N = 1], primary age-related tauopathy [PART, N = 1]), and limbic-associated TDP43 encephalopathy neuropathologic change (LATE-NC, N = 4). Pupil-optic nerve cross sections of bilateral fixed globes and sections of LGN were stained for beta-amyloid, hyperphosphorylated tau (p-tau), and phospho-TDP43.
Results: Scant beta-amyloid deposits were present in peripheral retina in 3 cases of AD-NC. These had morphologies different from cerebral neuritic or diffuse plaque and no staining/birefringence with Congo red. Diffuse plaques were present in LGN in 3 cases of AD-NC (2 with retinal aggregates). P-tau staining was present in peripheral retina, primarily inner plexiform layer, in 4 cases with AD-NC (2 with coexisting AGD and ARTAG). However, p-tau accumulation was not found in retinas of 4 other cases with cerebral tauopathies (FTLD-tau, ARTAG, AGD, and CTE). P-tau stain of LGN highlighted pretangles or neurites in 4 cases with cerebral tauopathy (2 with retinal aggregates), but staining was absent in 4 other cases of tauopathy (including 2 with retinal aggregates). No neurofibrillary tangles were present in retina or LGN. TDP43 deposits were present in peripheral outer plexiform layer of retina in 3 cases, all of which had AD-NC (1 with coexisting LATE-NC) but was absent in 3 other cases of LATE-NC. TDP43 was not identified in LGN.
Conclusions: The relationship between retinal, LGN, and other cerebral aggregates is complex, with differences in proteins present and morphology.

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An unusual case of combined true exfoliation and pseudoexfoliation of the lens

L Young, M Rolain, A Dirghangi, J Mandell, Z Karcioglu
University of Virginia

Background: Pseudoexfoliation syndrome is an age-related systemic disease mainly affecting ocular tissues with deposition of fibrillary proteinaceous material on the lens capsule, corneal endothelium, iris and ciliary body. True exfoliation is much more rare and is linked to chronic damage of the anterior lens capsule due to heat contact (ex. glassblowers) and exposure to infrared radiation.
Methods: An 81-y/o male presented for primary open angle glaucoma, right eye (OD) more involved than left (OS). He has been a glassblower for over 30 years, OD- dominant. Clinical findings were notable for 2+ nuclear sclerotic cataract with few vacuoles, peripheral peeling of the anterior lens capsule and subtle folds of the capsule were observed. Faint pseudoexfoliative rosette deposits were noted centrally in the anterior chamber. OS only demonstrated cataract without capsular changes. The diagnosis was likely pseudoexfoliation glaucoma with high suspicion for true exfoliation. He underwent a selective laser trabeculoplasty of the OD with improvement of intraocular pressure (IOP). One year later the IOP increased with cataracts becoming visually significant he underwent minimally invasive glaucoma surgery, goniotomy, endocyclophotocoagulation, cataract extraction with large intact anterior capsulotomy to evaluate for pathologic examination of the lens capsule.
Results: Histopathology was notable for eosinophilic proteinaceous deposits opposite the lens epithelium diagnostic of pseudoexfoliation. There was also early delamination of the anterior lens capsule at its anterior aspect. Transmission electron microscopy demonstrated thinning and incipient splitting of the anterior lens capsule and abnormal fibrils in the capsular layer, supportive of true exfoliation. The subcapsular lens epithelium (LE) cells displayed cell loss, shrinkage, pyknosis and intracellular-vacuoles.
Conclusions: This case is notable for exceedingly rare coexistence of true and pseudoexfoliation of the anterior lens which are generally considered to be etiologically distinct.

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Post-mortem pathologic findings in the eyes of patients with SARS-CoV-2 infection

M Marshall, M Lang-Orsini, A Stemmer-Rachamimov
Massachusetts General Hospital

Background: Coronavirus disease 2019 (COVID-19) has become a worldwide public health crises since its emergence in

late 2019. Infection by the SARS-CoV-2 virus most commonly leads to mild to moderate respiratory illness; however, systemic involvement and associated hyperinflammatory syndromes have been increasingly recognized. Pathologic studies to examine the involvement of the eyes by SARS-CoV-2 have been sparse, but reported pathologies associated with COVID-19 include conjunctivitis, retinal hemorrhage/ischemia, choroiditis, anterior uveitis, and optic neuritis.

Methods: Sections of formalin-fixed, paraffin-embedded eyes were examined from 21 patients (42 eyes) with a history of a confirmed SARS-CoV-2 nasopharyngeal swab positive test and from 10 control patients (20 eyes) that died in 2018 or 2019, prior to the outbreak of SARS-CoV-2. Patients with a history of COVID-19 infection were grouped into either having a remote infection (death >3 months after positive test and negative repeat post-mortem swab), acute infection (death < 3 months after positive test or positive post-mortem swab), or active covid-related pneumonia (positive post-mortem swab and histologic findings in lungs consistent with covid-related pneumonia). Immunohistochemical staining was performed for CD3, CD20, CD68, and CD163, and all cases were examined for the presence of SARS-CoV-2 RNA by in situ hybridization (ISH).

Results: This study identified a significantly higher incidence of inflammatory conditions of the eyes from COVID-19 positive patients (7/21) compared to controls (0/10) [$p = 0.039$; two-tailed z-test:-2.06158]. Identified pathologies included three cases of choroiditis, three cases of irido cyclitis, and one case of keratitis. All positive cases were identified within the acute/active populations. SARS-CoV-2 RNA was not detected by ISH in any patients.

Conclusions: These results identified a significant number of inflammatory conditions within post-mortem eye specimens from patients with SARS-CoV-2 infection. The number of cases studied here is larger than any previously reported pathology study and will help elucidate the incidence of SARS-CoV-2-related eye pathology.

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A Common Entity Presenting in a Unique Site

J Suddock[1], A Cuber[2], S Edminster[2], K Hurth[3], J Berry[3], M Sibug-Saber[3], A Mathew[3]
[1]University of Southern California + Los Angeles County Hospital; [2]University of Southern California + Los Angeles County Medical Center; [3]Keck School of Medicine of USC

Background: Conjunctival amyloidosis is a localized process rarely associated with systemic disease commonly occurring in middle-aged adults.

Methods: A 70-year-old woman presented with a one-year history of redness in her right conjunctiva. She also complained of discomfort and a gritty sensation. She was initially treated at an outside institution with topical agents without any improvement, following which she was referred to our institution. Slit lamp examination revealed an approximately 4 mm pink-white gelatinous lesion with abnormal vessels at 6-9 o'clock in the temporal aspect of the right eye. Anterior segment optical coherence tomography showed a primarily subepithelial mass with heterogenous areas and an intact underlying sclera. Given the clinical presentation, lymphoma was suspected, and a right conjunctival biopsy was performed.

Results: Three irregular, tan-white tissue fragments were received (0.2-0.7 cm) for histopathologic examination. A portion was submitted in RPMI for flow cytometry. Microscopic examination showed pale eosinophilic acellular material. In a few foci, these deposits appeared to be accentuated around blood vessels. A cellular infiltrate was not appreciated. Congo Red showed apple-green birefringence. The deposits were also positive with Amyloid P and did not stain with amyloid A. Transthyretin was equivocal.

Conclusions: Amyloidosis should be considered in the differential diagnosis of a patient with a 'salmon' colored lesion. Although conjunctival amyloidosis is rarely associated with systemic disease these patients should be worked up clinically to exclude systemic involvement.

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Massive Retinal Gliosis Mimicking an Intraocular Neoplasm

J Suddock[1], A Cuber[1], A Mathew[1], M Sibug-Saber[2]
[1]University of Southern California + Los Angeles County Hospital; [2]University of Southern California + Los Angeles County

Background: Massive retinal gliosis (MRG) is a diagnostic entity first described in 1905. Gliosis is the non-neoplastic proliferation of glial cells which can occur due to disruption of neuroglial and neuronal structure, function, and homeostasis. Histologically, it can mimic neoplastic entities with spindle cell morphology. MRG has been described in association with phthisis bulbi, trauma, congenital diseases or malformations, and retinal detachment. The lesion was recently further characterized into three subcategories based on the volume of the vitreous cavity that the tumor occupies.

Methods: We present a case of an 18-year-old woman with history of prosthetically managed congenital microphthalmia of the left eye who presented with persistent pain. Given the pain and discomfort, enucleation of the eye was recommended and performed.

Results: The enucleated globe was found to contain an intraocular mass occupying greater than 50% of the globe. Microscopic examination revealed interweaving groups of spindled cells and thick-walled, hyalinized vessels reminiscent of a schwannoma. The tumor cells had uniform elongated nuclei, abundant eosinophilic cytoplasm, and indistinct cell borders. Mitotic figures were not readily identifiable, and there were no areas of necrosis. Metaplastic bone and focal fibrous metaplasia were identified. Degenerative changes included calcifications, pigment-laden macrophages, cholesterol clefts, and cystic change. The mass was intimately associated with the retina. By immunohistochemistry, lesional cells were diffusely positive for S100, CD57, and GFAP. Lesional cells were negative for

melanocytic markers HMB45 and MelanA, for smooth muscle stains SMA and Desmin, and for an epithelial stain pankeratin. Ki-67 was low (< 5%). IDH-1 (R132H) was negative and P53 did not show over-expression.
Conclusions: MRG should be considered in the differential diagnosis for an intraocular spindle cell mass lesion, which includes schwannoma, glioma, and amelanotic melanoma. Although histology is similar, the entities can be differentiated by stains and supportive clinical history.

FRIDAY POSTERS: Peripheral Nerve/Muscle

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Novel Desmin Mutation in Head Domain Associated with Dysphagia and Hypotonia in an Infantile Onset Case

H Huang[1], Y Chen[2], W Weng[3]
[1]National Taiwan University Hospital; [2]National Taiwan University Cancer Center; [3]National Taiwan University Children Hospital

Background: Clinical features of myofibrillar myopathies related to pathogenic mutations of the human desmin (DES) gene include progressive muscle weakness, cardiomyopathy, cardiac conduction disease, and respiratory insufficiency, etc. Desminopathies are inherited as autosomal dominant or autosomal recessive trait, and the latter primarily present with muscle weakness in infants or children.
Methods: Here, we report a boy with an autosomal recessive desminopathy presenting with hypotonia, difficulty in swallowing, decreased muscle power, and flaccidity muscle tone since birth. During the infancy, persistent hypotonia, frequent choking, delayed motor developmental milestones, and elevated serum level of creatine kinase were noticed. An echocardiogram did not reveal evidence of cardiomyopathy or cardiac conduction disease. Video fluoroscopic swallow study revealed moderate to severe pharyngeal dysphagia as well as type I laryngeal cleft and laryngeal penetration. The boy underwent gastrostomy tube insertion and laryngoplasty at 18 months old. Thereafter, he received continuous oral neuromuscular training and thickened liquid diet to manage dysphagia.
Results: Right quadriceps muscle biopsy revealed marked variation in fiber size and frequent atrophic myocytes. Desmin expression was lost or significantly decreased in most fibers, but intense subsarcolemmal desmin staining was present in few myocytes. Electron microscopy did not demonstrate granulofilamentous material that is common for desminopathies. Instead, subsarcolemmal accumulation of abnormal large mitochondria, occasional Z-line streaming and myofibrillar disarray associated with Z-line fragmentation were noticed. Targeted next generation sequencing (NGS) revealed a novel frameshifting c.194dupG (p.Leu66fs) homozygous mutation in DES head domain, and the patient's mother possessed a heterozygous DES gene variation at the same point.
Conclusions: This case illustrated that dysphagia can be a major clinical problem in desminopathies and makes the diagnosis difficult to make. NGS serves as a utility tool, particularly in exceptional cases lacking characteristic deposition of granulofilamentous material.

FRIDAY POSTERS: Forensices/Trauma

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Association of Traumatic Brain Injury with Vessel Mineralization in Young Adult Men

R Mittenzwei, D Keene, C Latimer, H Maioli, D Marshall, A Nolan, K Scherpelz
University of Washington

Background: The chronic neuropathologies that develop after traumatic brain injury (TBI) and may be associated with an increase in the risk of dementia and neurodegenerative disease are research topics of great interest to the community. Many studies have focused on pathologies in older-aged cohorts, in which aging and neurodegenerative pathologies may obscure the initial inciting mechanisms. Recently, vascular pathologies have been associated with TBI in such a cohort. Certainly, animal models, even of mild TBI, also suggest a role for vascular pathology with frequent blood brain barrier disruption.
Methods: Our goal was to investigate whether vascular pathology might already be present and associated with TBI in a younger cohort of brain donors enriched for mild community-associated TBI, reducing the confounding effects of aging-related pathologies. The brains of male donors in our Pacific Northwest Brain Donor Network under 60 years of age (average age = 44) were evaluated across several vascular metrics including atherosclerosis, arteriolosclerosis, macroscopic infarcts, microinfarcts, and basal ganglia vessel calcification.
Results: There was a notable difference in pathology with TBI compared to controls with basal ganglia vessel calcification ($p = .0167$), which was identified in 32% of donors with a history of TBI (several in their 20s), but none of the donors without TBI.
Conclusions: This is a striking finding and supports additional research into the role of chronic vascular changes after TBI in inciting progressive pathology.

FRIDAY POSTERS: Tumors: Glial

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Age-spectrum of CNS tumors in a low-middle income country: Comparison with the US national tumor registry

A Gilani[1], M Shakir[2], A Altaf[2], S Saqib[2], Z Siddiq[3], S Enam[3]
[1]University of Colorado, Aurora Colorado;The Aga Khan University, Karachi, Pakistan; [2]Aga Khan University; [3]The Aga Khan University

Background: Pakistan, like many other low-middle-income countries, lacks a national tumor registry, hence the incidence

of CNS tumors, age-spectrum and the relative frequencies of various tumor types are unknown. In this population, consanguinity rate approaches 65% but the frequency of CMMRD-related tumors and overall effect on tumor distribution is unknown. >50% of the country's CNS tumor cases are being reviewed in our clinical practice. We use this data to estimate the age-spectrum and relative frequencies of various CNS tumors in Pakistan. We hypothesize that there is a higher incidence of pediatric neuroepithelial tumors in Pakistan relative to the US.

Methods: Cases received for histopathologic diagnosis by one of the authors (AG) between 9/2022 and 1/2023 were reviewed. Clinical, radiologic and histopathologic diagnoses were recorded.

Results: 84% (440/534) of the cases were neoplastic, out of which 239 were neuroepithelial. 24% of all neuroepithelial tumors were in children (0-14 yrs), with AYA (adolescent/young adult) (15-39 yrs) and adults (40 years and older) accounting for 37% and 38% respectively. The age distribution of neuroepithelial tumors in SEER database (published 2022) was skewed towards an older age (Children: 11%, AYA: 16%, older adult: 72.5%). As compared to the US, in Pakistan, pediatric cases represent a significantly larger proportion of all neuroepithelial tumors. This difference can however be explained by the greater representation of this age group in the Pakistani population. The average annual age-adjusted incidence rate (per 100,000 population) for US population is Children 4.19 (CI 4.12-4.26), AYA 3.46 CI:3.41-3.52), adults 10.30 CI (10.23-10.37). This rate is unknown for Pakistan.

Conclusions: Relative age distribution of neuroepithelial tumor rate is not significantly different between the Pakistani and US populations indicating that despite high consanguinity, there is low prevalence of CMMRD and other genetic tumor predisposition syndromes.

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Characterization of Central Nervous System Tumors with a Near-Haploid Genome

R Elhalaby, T Kollmeyer, S Jenkins, C Ida, C Giannini, A Raghunathan, J Trejo-Lopez, A Nguyen, C Zepeda Mendoza, R Jenkins, R Vaubel
Mayo clinic

Background: Near-haploidy is an unusual phenomenon occurring in a variety of cancers, including giant cell glioblastoma.

Methods: We have characterized the histologic and molecular features of 36 central nervous system tumors identified on chromosomal microarray to have a near-haploid genome. Diagnoses included glioblastoma, IDH-wildtype (n = 23), astrocytoma grades 2-4 NOS/NEC (n = 8), diffuse pediatric-type high-grade glioma, IDH-wildtype and H3-wildtype (n = 1), pleomorphic xanthoastrocytoma (n = 1), atypical meningioma (n = 2) and atypical teratoid rhabdoid tumor (n = 1).

Results: Median age for glioma patients (n = 33) was 40 years (range 13-87), 54.5% were males, and the frontal lobe was involved in 48.5%. Fourteen glioblastoma patients (60.8%) were younger than 55 years (median 40 years) including 2 pediatric patients (8.7%). Only 4 glioblastomas (17.4%) showed predominant giant cell morphology while 17.4% had minor giant cell component and the remaining cases (65.2%) showed insignificant giant cell population with predominant fibrillary, epithelioid or spindle morphology. Chromosomal microarray showed marked loss of heterozygosity (LOH) in all cases, involving from 9 up to 22 chromosomes. All gliomas showed relative gain of chromosome 7 and at least partial LOH of chromosome 10. Other common gains involved chromosomes 5, 21 (23/33, 69.7%) and 20 (19/33, 57.6%). CDKN2A/B homozygous deletion was present in 9 cases (27.3%), CDK4 and PDGFRA amplifications were present each in one case (3%) while no cases showed EGFR amplifications. Additional molecular testing included targeted next generation sequencing (n = 24), IDH1/IDH2 sequencing (n = 7), TERT promoter sequencing (n = 6). The most common mutations included TP53 (19/24, 79.2%), NF1 (12/24, 50%), RB1 (10/24, 41.7%) and PTEN (8/24, 33.3%). Other less frequent alterations included BRAF, ATRX, CHEK2, TSC2, NOTCH1 each in 2/24 cases (8.3%) and TERT (2/30, 6.7%).

Conclusions: Near-haploidy can occur in a range of CNS tumors, most frequently in glioblastoma with or without giant cell morphology. These glioblastoma patients are younger and enriched for tumors harboring TP53, NF1, and RB1 mutations.

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In vivo confocal laser endomicroscopy in brain tumors: "Live" neuropathological evaluation at tumor center and margins

B Pollo[1], F Restelli[2], E Mazzapicchi[2], M Patanè[2], C Calatozzolo[2], I Tramacere[2], M Broggi[2], M Schiariti[2], P Ferroli[2], F Di Meco[2], F Acerbi[2]
[1]Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy; [2]Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy

Background: In vivo confocal laser endomicroscopy (CLE) is a new intraoperative cellular-resolution fluorescence-based imaging technology that permit a microscopic analysis in near-real time and may have a significant impact on resection strategies for brain tumors. The main purpose of our study was to validate the CLE ability in correctly identifying histological structures in different types of Central Nervous System (CNS) tumors during neurosurgical procedures, at tumor center and at the margin of resection, specifically in the infiltrating high-grade gliomas (HGGs) to improve tumor removal.

Methods: We carried out a clinical trial on 75 consecutively enrolled patients with suspected HGGs and aggressive CNS tumors between 2020 and 2022, comparing the results of multiple neuropathological and CLE virtual biopsies at tumor center and at margins. Our cases comprehended glioblastoma (50,0%) and metastasis (11,3%), other gliomas, ependymomas and some rare tumor.

Results: CLE images revealed features similar to tissue architecture/morphology seen in standard histology, with good correlation in matched histological images. At the tumor center we obtained a total of 148 virtual biopsies, with a 67.2 ± 6.0 % of complete/partial accuracy in offering an intraoperative diagnosis. On the HGGs subset with CLE a correct identification of gross morphological features was achieved in all the cases. At the tumor periphery, a total of 144 virtual biopsies were obtained, of which 76 in fluorescent tissue and 68 in not fluorescent tissue, with tumor tissue identification at fluorescent margins as 93.5 ± 3.1 % and 94.6 ± 3.7 % considering only HGGs.
Conclusions: CLE may have a role in intraoperative diagnosis with an evaluation of neoplastic tissue at the tumors center, and can detect presence or absence of tumor infiltration at margins. Our results suggest an important application for intraoperative CLE imaging in neurosurgery, providing histological information to improve extent of resection in CNS tumors and mainly in HGGs.

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Routine Comprehensive Solid and Fusion Genetic Profiling Uncovers Novel Fusions in Pediatric and Young Adult Brain Tumors

M Gubbiotti[1], M Luo[2], M Nasrallah[1], S Stone[1], M Santi[2], A Viaene[2]
[1]Hospital of the University of Pennsylvania; [2]Children's Hospital of Philadelphia

Background: We routinely assess all brain tumors for genetic alterations for diagnostic and treatment purposes. As our institution's extensive fusion transcript panel targets over 700 exons of 117 genes, we hypothesized that novel gene fusions were uncovered during regular workflow.
Methods: We analyzed all gene fusions in pediatric and young adult brain tumors sequenced via Children's Hospital of Philadelphia (CHOP) Comprehensive Solid Tumor Panel between 2016-2022.
Results: We analyzed 1110 brain tumors (802 pediatric, 436 male, 366 female, average age: 11.1 years and 308 adult, 157 male and 146 female, average age: 45.2 years) and identified 248 gene fusions (211 pediatric, 26% and 37 adult, 12%). The most common fusion within pediatric tumors was KIAA1549::BRAF. Fusions were less often found in adult tumors. Common partners included FGFR and BRAF. Seven novel/unusual fusions were identified (6 pediatric, 1 adult), which were separated into three groups: 1. Novel fusions appropriate for tumor category: dysembryoplastic neuroepithelial tumor with FGFR3::FAM184B, pilocytic astrocytoma with GNAI2::BRAF, and two glial/glioneuronal tumors with JAM3::BRAF and ARIH1::BRAF (adult tumor) alterations; 2. Unusual fusions not typically associated with a particular tumor entity: pleomorphic xanthoastrocytoma with TPM3::NTRK1 and extraventricular neurocytoma with KIF5B::NTRK2; 3. Novel fusion in an unclassifiable tumor: high-grade neoplasm with BRD4::LEUTX. All patients had low-grade tumors with excellent outcomes except the patient with BRD4::LEUTX-fused tumor who died five years following initial diagnosis.
Conclusions: We conclude Next Generation Sequencing performed as part of the clinical workup can identify novel/rare fusions. While the biologic and prognostic implications of these alterations are uncertain, the identification of novel fusions expands our knowledge of genetic signatures associated with both known and previously undescribed tumor entities and may offer therapeutic targets for future treatment.

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High-grade Glioma with Pleomorphic and Pseudopapillary Features (HPAP) in the Setting of Li-Fraumeni Syndrome and with Extracranial Metastasis

K Hiatt[1], K Aldape[2], M Kumar[3], N Massoll[3], M Gokden[3]
[1]Department of Pathology, University of Arkansas for Medical Sciences; [2]National Institutes of Health/National Cancer Institute; [3]University of Arkansas for Medical Sciences

Background: HPAP is a recently-described, well-circumscribed glioma with heterogeneous histologic features mimicking a variety of gliomas, frequent TP53, RB1, NF1, and NF2 mutations, and losses of chromosomes 13 and 17. Li-Fraumeni syndrome (LFS) is characterized by germline TP53 alterations and neoplasms in multiple organs, including brain. Extracranial metastases are rare in brain tumors. Here, we report a case with these features, which, to our knowledge, has not been reported in the English literature.
Methods: Case Presentation
Results: A 24-year-old man with headaches and mild aphasia had a 5.4 cm left parietal heterogenous, peripherally-enhancing mass, showing a well-circumscribed glioma with pseudorosettes, pleomorphism, giant cells, and areas with diffuse glioma appearance, variable positivity for GFAP, S-100 protein, and olig2, dot-like positivity for EMA, and high Ki-67 proliferation index. NGS identified alterations of NF1, RB1, TSC2, GPS2, and TP53, as well as germline mutation of TP53. Pertinent negatives included mutations of IDH 1 & 2, ATRX, and BRAF V600E, EGFR amplification, TERT promoter mutation, and MGMT promoter methylation. It matched to methylation class HPAP. He had a diagnosis of multiple juvenile polyposis coli with adenomatous changes, status post colectomy at two years of age; mismatch repair proteins were intact. After the initial diagnosis and chemo-radiation, he had multiple additional lesions, recurrences, and surgeries, recent specimens with more monotonous histology. Finally, multiple neck masses developed. Fine-needle aspiration cytology identified a metastatic high-grade glioma in a lymph node. He expired 32 months after initial diagnosis.
Conclusions: Brain tumors can be a component of various syndromes, some of which may have overlapping combinations, e.g., LFS, familial adenomatous polyposis 1, and constitutional mismatch repair deficiency syndrome. Thorough clinical, radiologic, and pathologic examination with molecular-genetic work-up are crucial for accurately characterizing these

cases, shedding light on obscure features, as in this case where an HPAP and LFS with extracranial metastasis are identified.

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Comparative assessment of methodology in the detection of MGMT promoter methylation in FFPE samples

M Mrdelja, L Li, K Kelly, G Omerza, N Renzette, M Soucy, X Zhuo, V Induri, R Gorham
The Jackson Laboratory for Genomic Medicine

Background: DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) gene promoter methylation is used as a clinical biomarker in glioma and glioblastoma patients to predict response to treatment with alkylating chemotherapy, such as temozolomide, and is indicative of favorable prognosis. Since the current standard of care involves temozolomide chemotherapy, there is significant clinical benefit in a reliable screening method to identify patients that will or will not respond to treatment. Currently, several methods exist to detect MGMT promoter methylation, but there is debate over which is the most appropriate method. Therapeutic determination according to tumor MGMT status requires a validated, standardized diagnostic test, suitable for the analysis of formalin-fixed, paraffin-embedded (FFPE) tissue.
Methods: In 2022, 2 methods for analysis of methylation status were validated by the CLIA laboratory: 1) JAX MGMT Promoter Methylation, a methylation-specific PCR with High Resolution Melt (HRM) analysis, and 2) JAX OncoMethyl Array, a genome-wide methylation screening array (Infinium MethylationEPIC v1.0 BeadChip Kit, Illumina). Test samples were processed and analyzed using both methodologies, and results were compared.
Results: Among 43 FFPE samples processed, 40 samples (~93%) evaluated by methylation array were concordant with the methylation-specific PCR methodology. All 3 discordant were classified as Intermediate methylation on the HRM method as compared to methylated classification on the array.
Conclusions: The two different methodologies are compared for their advantages and disadvantages in clinical practice. The detection of MGMT promoter methylation status and associated clinical interpretations in CNS tumors are comprehensive, pending for additional clinical correlation studies.

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Melanin Production in a Myxopapillary Ependymoma, Atypical
Histology and Unusual Molecular Findings: A Case Report

J Afude, H Takei, B Guthikonda, L De Alba Padilla, A CuevaS Ocampo
LSU Health Shreveport

Background: Myxopapillary ependymoma (ME) is a rare glial neoplasm with an incidence of 0.6 to 1.0 cases per 1 million person-years and a relatively favorable prognosis. ME commonly affects the conus medularis and filum terminale. Histologically, ME reveals a prototypical papillary pattern with hyalinized fibrovascular cores, myxoid material around blood vessels and microcysts. Melanin pigment is rarely expressed by glial neoplasms and even more rare is pigmented conventional ependymomas. To our knowledge, melanin pigmentation in ME has not been described in the literature.
Methods: we report a healthy 29-year-old male presenting with chronic back pain, lower limb paresthesia, and a well-defined solid mass in the conus medullaris on MRI.
Results: Histopathology revealed a spindle to epithelioid neoplasm with compact architecture admixed with loose areas containing spherical mucin-rich microcysts. The spindle cell component exhibited minimal pleomorphism and foci of melanin production. Atypical features including necrosis, microvascular proliferation, or increased mitoses were not identified. Immunohistochemistry showed positivity for S100, GFAP, CD99, AE1/AE3, CD56, and synaptophysin dot-like positivity. The melanin producing cells were weakly positive for SOX10, S100, MART1, and HMB45 and positive for Fontana-Masson. The tumor cells were negative for iron stain, IDH1, EMA, OLIG2, and CD34. The Ki67 proliferation index was estimated at 2%. Chromosomal microarray analysis (CMA) showed gains of chromosomes 4, 5, 7, 9, 12,15, 16, 17, 18, 19 and 20, and loss of heterozygosity of chromosomes 8, 10, 13, and 14. Next generation sequencing (NGS) identified pathogenic mutations on ATM (c 131A>G) and MYC (c 1307G>A) of uncertain significance.
Conclusions: These features were consistent with ME with melanin production. This finding is extremely rare, and we want to raise awareness of its occurrence to avoid diagnostic pitfalls. Immunohistochemistry, histochemistry, CMA, NGS, and methylation profiling (if available) are useful tools to narrow down the differential diagnosis when encountering this phenomenon.

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Long survival of a young adult with a diffuse hemispheric glioma, H3 G34-mutant

M Basuino[1], S Litofsky[1], J Cousins[1], R Jenkins[2], C Ida[2], D Miller[1]
[1]University of Missouri; [2]Mayo Clinic

Background: Diffuse hemispheric glioma, H3 G34-mutant (DHG, H3 G34) is a new WHO 2021 grade 4 tumor type reported to carry a dismal prognosis with mean survival ranging from 18 to 22 months. We present a patient with a DHG, H3 G34 who has survived 9.5 years after initial tumor resection.
Methods: A 26-year-old man with no prior neurologic history presented with seizures in October 2013. MRI showed a minimally enhancing T1-hypointense 2.0 x 1.4 x 1.5 cm left frontal mass without surrounding edema. Gross-total resection was performed with histological diagnosis of an anaplastic oligodendroglioma, WHO Grade III. Molecular testing at that time showed the tumor was IDH-wildtype (targeted

pyrosequencing) without 1p/19q co-deletion (FISH). He received neoadjuvant radiation and temozolomide chemotherapy for one year. In July 2022, MRI/MRS showed new contrast enhancement within the splenium and bilateral parietal and occipital white matter which prompted additional molecular testing of the original tumor. The patient continues to be minimally symptomatic.

Results: Targeted neuro-oncology NGS testing revealed an H3 G34R mutation [H3-3A:c.103G>A p.Gly35Arg] and mutations in TP53 (x2), ATRX, and PDFGRA; the tumor was reclassified as a DHG, H3 G34. Chromosomal microarray showed a complex copy-number profile, with more than 20 alterations and 20% of the genome altered, including loss with loss of heterozygosity of 3q, 4q and 9p (encompassing CDKN2A/CDKN2B); no oncogene amplifications were observed. The MGMT promoter was methylated (RT-PCR assay interrogating 8 downstream CpG sites). Histological re-review showed a mitotically active infiltrating glioma with pleomorphic giant cells and a primitive neuronal component, without necrosis or microvascular proliferation.

Conclusions: To our knowledge, this is the first case of long-term survival of a patient with a DHG, H3 G34. While this patient's tumor had prognostically favorable MGMT promoter methylation and lacked unfavorable oncogene amplifications, the potential mechanisms for such prolonged survival remain to be determined.

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Fusion Detection in Primary Neoplasms of the CNS Using the TSO500 NGS RNA/DNA Hybrid Panel

J Zeitlin, B Liechty, J Solomon, D Pisapia
Weill Cornell Medicine

Background: Diagnostic use of next generation sequencing (NGS) panels that employ anchored multiplex PCR has increased the rate of fusion transcript detection in our neuropathology practice. Using the internally validated TruSight™ Oncology (TSO500) panel that allows for partner-agnostic fusion detection, we describe the rate of fusion calls in a series of primary CNS neoplasms that includes both diffuse gliomas and other neuroepithelial tumors.

Methods: 167 distinct cases were sequenced using the Illumina TSO500 targeted hybrid-capture based NGS assay. RNA is extracted from FPE tissue using the Allprep DNA/RNA FFPE Kit (Qiagen, Inc.). RNA is then reverse transcribed to cDNA for fusion detection. The regions of interest are hybridized to biotinylated probes. Libraries are normalized using a bead-based protocol, pooled, and sequenced on an Illumina NextSeq 500 instrument.

Results: Fusion transcripts were detected in 38 of 167 cases (23%). In 65% (15/23) of histological pilocytic astrocytomas, detected fusionsincluded: KIAA1549-BRAF (n = 13); GTF2I-BRAF (n = 1); and FGFR1-NKAIN3 (n = 1). In 17% of 83 glioblastoma cases, we detected at least one fusion, including: FGFR3-TACC3 (n = 3); PTPRZ1-MET (n = 4); and one each of IFRD1-MET, CAPZA2-MET, and KANK2-NTRK2 among others. Fusions involving FGFR2 with either NRIP3 or KIAA1598 were seen in 2 cases of PLNTY, while an additional 2 cases of neuroepithelial tumors (NOS) showed FGFR2- fusions with either CTNNA3 or LRRFIP1. Additional fusions included an NTRK2 fusion in an IDH-mutant astrocytoma and a QKI-RAF1 fusion as a putative driver of an astrocytoma, NOS.

Conclusions: With increased use of fusion partner-agnostic methods, a broader range of potential oncogenic drivers can be detected. In addition to common partners (e.g. KIAA1549 for BRAF) we detected less common partners to BRAF including GTF21 as well as less common partners for MET and NTRK2 fusions in glioma. Future studies are needed to determine the full diagnostic utility and therapeutic implications of expanded fusion detection.

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IDH-mutant astrocytomas with primitive neuronal component are aggressive tumors characterized by alterations in RB1 and TP53

C Slocum[1], R Hickman[2], E Gedvilaite[2], R Ptashkin[2], R Brannon[2], M Ladanyi[2], A Miller[2], E Pentsova[2], V Tabar[2], R Young[2], I Mellinghoff[2], M Rosenblum[2], T Bale[2]
[1]New York Presbyterian-Weill Cornell Medicine; [2]Memorial Sloan Kettering Cancer Center

Background: Glioblastomas with a primitive neuronal component, in addition to their distinct histologic appearance, have recently been demonstrated to harbor a characteristic molecular signature, including a unique methylation profile and frequent alterations of RB1, PTEN, and TP53, distinguishing them from other IDH-wildtype glioblastomas. Indeed, loss of RB1 function/expression associated with the acquisition of a "small cell" neuroendocrine phenotype is a progression-related phenomenon potentially displayed by a variety of solid tumors. While primitive neuronal morphology has been described in IDH-mutant astrocytomas, the associated molecular features of this rare morphologic pattern are not well understood.

Methods: From a cohort of 676 IDH-mutant gliomas characterized by a targeted hybrid capture next-generation sequencing assay (341-505 cancer genes, MSK-IMPACT), we identified 26 IDH-mutant astrocytomas with deleterious alterations in RB1 for histologic review. Additional clinicopathologic information was retrieved from electronic medical records.

Results: Among IDH1/RB1 co-mutant astrocytomas, 50% (13/26) of the cases had a well-defined primitive neuronal component; all of which also met 2021 histologic criteria for CNS WHO grade 4. 100% of cases with primitive neuronal component also demonstrated mutations in TP53 (13/13) while only 7.7% (1/13) had a mutation in PTEN. Additional recurrent alterations included ATRX mutations (84.6%, 11/13), ERCC5 gain (30.8%, 4/13), CDKN2A/2B loss (23.1%, 3/13) and focal amplifications of CDK4 (23.1%, 3/13), MET (15.4%, 2/13), PDGFRA (15.4%, 2/13), and MYCN (15.4% 2/13). Median overall survival was 36.8 months, varying from 7.6 to 211 months after initial diagnosis. Among IDH1/RB1

co-mutant astrocytomas, the presence of a primitive neuronal component was associated with significantly shorter overall survival (P = 0.022, hazard ratio [HR]: 9.00, 95% CI: 4.17–19.4).
Conclusions: IDH1/RB1 co-mutant astrocytomas frequently demonstrate a primitive neuronal component, which is associated with characteristic mutations and a highly aggressive disease course.

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Ultrasensitive ddPCR Detects Evidence of MGMT Promoter Methylation in all Glioblastoma Samples from Within and Beyond the Surgical Margin

E Goold[1], S Hellwig[2], A Matynia[3], C Dean[3], R Malani[3], B Harrison[3], M Bronner[3], H Colman[3], R Jensen[3], H Underhill[3]
[1]University of Utah and ARUP laboratories; [2]ARUP Laboratories; [3]University of Utah

Background: In IDH wild-type glioblastoma with evidence of MGMT promotor (MGMTp) methylation, use of temozolomide has a proven survival benefit. Current methods to determine MGMTp methylation status have reported analytical sensitivities ranging from 1.5-5%. Here, we validated an MGMTp methylation assay with a sensitivity of 0.1% to study tissues within and beyond the surgical margins of glioblastoma.
Methods: Bulky tumor (BT) and peritumoral edema (PE) samples acquired from newly diagnosed adult glioblastoma patients were snap-frozen followed by bisulfite conversion of extracted DNA. A droplet digital PCR (ddPCR) assay was developed to analyze MGMTp methylation at CpGs 75-82 with a 0.1% analytical sensitivity, 0.5% limit of quantitation, and >99% specificity. A TERT promoter ddPCR assay determined tumor cell percentage for normalization of MGMTp methylation.
Results: Twenty-seven BT and 23 PE samples were obtained from 14 patients (age: 31–88 yrs; 50% male). We detected the presence of MGMTp methylation in 100% (50/50) of BT and PE samples, which included patients previously classified as unmethylated by clinical standards (5/14). After normalization for tumor cell content, we found the extent of MGMTp methylation was similar in BT and PE (38.19±5.8% vs 50.4±7.6%, respectively; p = 0.21). Notably, in one patient classified clinically as unmethylated, we measured the normalized MGMTp methylation level at 11.05% in PE, reaching the clinical threshold of 5%, while the normalized MGMTp methylation level remained below 5% in the corresponding BT.
Conclusions: MGMTp methylation was present in all samples using our ddPCR approach. One clinically unmethylated patient demonstrated clinically actionable levels of methylation in PE suggesting that invasive subclones may be present in PE of clinically unmethylated patients with significant MGMTp methylation. Additional studies are warranted to determine if MGMTp methylation status in PE is an indicator of response to temozolomide.

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Molecular profiling reveals acquired increased tumor mutation burden in a subset of recurrent post-treatment pediatric high-grade gliomas

M Wood[1], C Beadling[2], T Neff[2], C Harrington[1], S Moore[2], L Baird[3], C Corless[2]
[1]Oregon Health & Science University; [2]Knight Cancer Institute, OHSU; [3]Boston Children's Hospital

Background: Adult-type diffuse gliomas can show markedly increased tumor mutation burden (TMB) at recurrence compared to the pre-treatment tumor, often related to temozolomide treatment and acquired inactivating mutations in the DNA mismatch repair (MMR) genes. To determine the relevance of this phenomenon in pediatric patients, we studied 11 pediatric high-grade gliomas with paired pre- and post-treatment tumor samples that were treated with temozolomide.
Methods: Institutional records were reviewed and 11 pediatric patients were identified with a high-grade glioma (non-ependymoma), with ≥ 2 pathology specimens and any intervening temozolomide treatment. Pre- and post-treatment samples were evaluated with MMR protein immunohistochemistry, next-generation sequencing (NGS), and genome-wide DNA methylation-based profiling.
Results: The 8 male and 3 female patients had median age of 12 years at diagnosis (range 6-17). Eight tumors were located in cerebral hemispheres, 2 were supratentorial midline, and one was cerebellar. Modernized integrated diagnoses were: diffuse midline glioma H3 K27-altered (DMG-K27) [N = 3], diffuse pediatric-type high-grade glioma H3/IDH-wildtype (pHGG-WT) [3], diffuse hemispheric glioma H3 G34-mutant (DHG-G34) [2], pleomorphic xanthoastrocytoma grade 3 (PXA) [1], astrocytoma IDH-mutant grade 4 [1], and high-grade astrocytoma NEC [1]. Three of 9 cases with paired NGS results showed increased TMB at recurrence. Two of these were DHG-G34 with recurrent tumor being spatially distinct from the primary site, with evidence for MMR gene mutations and loss of corresponding protein immunoreactivity private to the recurrence only (one previously published as PMID 33296093). The third was a case of pHGG-WT that lacked an identifiable molecular driver, MMR alteration, and definitive DNA methylation-based classification.
Conclusions: Post-treatment increased TMB was identified in one-third of the pediatric high-grade gliomas in this institutional cohort, including two cases of DHG-G34. Further studies may be warranted to determine if this tumor type is more susceptible to treatment-associated MMR deficiency

compared to other forms of pediatric-type diffuse high-grade glioma.

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Concurrent ependymal and ganglionic differentiation in a subset of Supratentorial Neuroepithelial Tumors with EWSR1-PLAGL1 rearrangement

J Lee[1], L Furtado[2], G Rose[3], T Larkin[4], R Sances[5], B Kleinschmidt-DeMasters[6], J Bodmer[6], M Gokden[7], K Bertrand[2], D Moreira[2], G Robinson[2], D Ellison[2], B Orr[2]
[1]St. Jude Children's Research Hospital; [2]St. Jude Children's Research Hospital, Memphis, TN; [3]Department of Pathology, St. Joseph's Hospital Tampa, FL; [4]Department of Pediatrics, Division of Neuro-Oncology, St. Joseph's Hospital Tampa, FL; [5]Department of Pathology, East TN Children's Hospital, Knoxville TN; [6]Department of Pathology, University of Colorado, Denver, CO; [7]Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR

Background: Neuroepithelial tumors with fusion of PLAGL1 or amplification of PLAGL1/PLAGL2 have recently been described often with ependymoma-like or embryonal histology respectively (PMID: 34355256, 36437415, 29539639).
Methods: To further evaluate emerging entities with PLAG-family genetic alterations, the histologic, molecular, clinical, and imaging features were described for a cohort of cases encountered clinically at St. Jude.
Results: Among six patients and ten resections, five supratentorial neuroepithelial tumors were identified with EWSR1-PLAGL1 fusion detected by RNA sequencing and a corresponding methylation profile of neuroepithelial tumor PLAGL1 fused (DKFZ 12.5). Additionally, one cerebellar mass was encountered with PLAGL2 amplification in a 2-year-old female. PLAGL1-fused cases (3F:2M) ranged in age from 9 months to 14 years at time of initial diagnosis. Three PLAGL1 fusion cases demonstrated ependymal and ganglionic features on initial resection, with a fourth case showing subclonal INI1 loss and developing a ganglionic component on recurrence. The PLAGL2 amplified case demonstrated embryonal histology. Clinical follow-up was available for 3/5 patients with PLAGL1 fusion (range: 11 months to 9 years) with various treatment modalities and extent of resection. Two patients developed recurrence, one at 7 months following treatment per ACNS0334 with intracranial multifocal disease and death at 11 months, and one patient with two recurrences at 1.6 years and 9 years after chemotherapy. Outcome data was not available for the PLAGL2 amplified tumor.
Conclusions: A histologic feature observed in a subset (4/5) of supratentorial neuroepithelial tumors with EWSR1-PLAGL1 rearrangement is the presence of ependymal and ganglionic differentiation. Though not present in all cases and unlikely to be specific, the combined presence of ependymal and ganglionic features may raise consideration for a Supratentorial neuroepithelial tumor EWSR1-PLAGL1 fusion, and prompt initiation of appropriate molecular testing such as RNA sequencing and methylation profiling. Continued compilation of associated clinical data will be critical for understanding emerging entities with PLAG-family genetic alterations.

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Pediatric type diffuse high grade glioma, H3-wildtype and IDH wildtype: Two adult cases with interesting histopathologic and molecular findings

M Elnagdy[1], G Kleinman[2], S Ahuja[2]
[1]Westchester Medical Center/NYMC; [2]Westchester Medical Center

Background: Pediatric-type diffuse high-grade gliomas (HGG) are a distinct group of glial tumors newly classified in the fifth edition of WHO classification. Pediatric type diffuse HGG, H3-wildtype and IDH-wildtype has very limited epidemiological data with median age of 3-18 years. The data about the incidence and the median age in adults is still lacking. Histopathologically, it shows either glioblastoma features or primitive undifferentiated morphology. Molecularly, it shows a characteristic methylation profiling which is one of the key diagnostic elements of this entity. We are reporting two cases of pediatric-type HGG H3-wild type and IDH-wildtype in a 28- and 46-years old patients with interesting histopathological features including extracellular mucin.
Methods: H&E staining for representative tumor sections was done according to our laboratory protocol. Immunohistochemistry was done using: GFAP, Ki67, p53. The cases were sent out for methylation profiling at National Institute of Health/National cancer Institute (Bethesda, MD). Expanded neuro-oncology panel was done in Mayo clinic laboratories (Rochester, MN).
Results: H&E sections show an infiltrative densely cellular tumor of small cells with round nuclei, indistinct nucleoli and scant cytoplasm. Mitotic figures are seen with microvascular proliferation. Cortical infiltration is seen with formation of secondary structures including perineuronal satellites and subpial aggregates. Extracellular mucin was observed highlighted by PAS-AB stain. The tumor was positive for GFAP and p53 with a high ki-67 labelling index. Molecular tests showed: IDH1/2 wild type, ATRX wild type, H3-wild type, negative EGFR amplification and positive MGMT promotor methylation. Methylation profiling was consistent with diffuse pediatric-type HGG, RTK1 subtype.
Conclusions: In this study, we are describing two cases of the novel entity, Pediatric type diffuse HGG, H3-wildtype and IDH-wildtype. The two cases are adult patients with characteristic histopathological findings. Also, we are showing their

expanded neuro-oncology panel and the methylation profiling. More case studies are needed for further characterization of this tumor.

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The utility of methylation profiling in the diagnosis of pediatric BRAF mutant glial and glioneuronal tumors

M Alturkustani
King Abdulaziz University, Jeddah, Saudi Arabia

Background: The differential diagnosis of BRAF mutants glial and glioneuronal is broad and encompasses CNS WHO grade 1-4 tumors. The specific diagnosis of these tumors can be challenging in a subset of these cases. Methylation profiling (MP) is a robust test for classifying CNS tumors and is recommended in unresolved cases. This work evaluates the benefit of MP in this setting.
Methods: Cases diagnosed as glial or glioneuronal tumors with BRAF mutation were retrieved from the children's brain tumor network (CBTN) online database. The initial cohort included 50 cases (56 samples). The inclusion criteria are the availability of the initial diagnosis or the scanned representative images, next-generation sequencing data, and the methylation profile file. The Heidelberg methylation brain tumor classifier (v12.5) was used through (https://www.molecularneuropathology.org/mnp) to get the MP results.
Results: Forty-six cases met the inclusion criteria (24 males, 22 females, age ranges four months to -20 years with an average of 9.9 years). The MP was confirmatory in 23 cases [12 pilocytic astrocytoma (PA), four gangliogliomas (GG), and seven pleomorphic xanthoastrocytomas (PXA)]. The diagnoses were modified in 2 cases [diffuse leptomeningeal glioneuronal tumor (DLGNT) and polymorphous low-grade neuroepithelial tumor of the young (PLNTY)], debatable in 7 cases [6 PA vs. GG and a desmoplastic infantile astrocytoma vs. PA], disregarded in 5 cases [2 high-grade gliomas (MP: PXA), two low-grade astrocytomas (MP: control tissue) and a possible GG (MP: cranial & paraspinal nerve tumor)], and non-contributary (calibration score less than 0.9) in 9 cases [4 GG (MP: control), two high-grade gliomas, three glia/ glioneuronal tumors (GNT)].
Conclusions: In BRAF mutants glial or glioneuronal tumors, MP modified the diagnosis in 2/46 cases and raised the debate of GG vs. PA in a subset of cases. However, it did not resolve the challenging cases, and the MP of PXA should be interpreted with caution.

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Astrocytoma, IDH-mutant: Is There a Role for Histologic Grading?

H Ozkizilkaya[1], H Ozkizilkaya[2], K Lam[2], A Dono[3], O Basit Ogunbona[2], D Wang[2], P Goli[2], E Wang[2], G Fuller[2], C Patel[2], C Kamiya Matsuoka[2], Y Esquenazi[4], L Ballester[2]
[1]The University of Texas MD Anderson Cancer Center Translational Molecular Pathology Department; [2]The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [3]The University of Texas Health Science Center at Houston, Houston, Texas, USA; [4]The University of Texas Health Science Center at Houston, Vivian L. Smith Department of Neurosurgery, Houston, TX, USA. Center for Precision Health, School of Biomedical Informatics, The University of Texas Health Science Center at Houston, Houston, TX, USA. Memorial Hermann Hospital-TMC, Houston, TX, USA

Background: The 2021 WHO classification of CNS tumors incorporates molecular alterations in the diagnosis and grading of diffuse gliomas. The 3 main types of adult diffuse gliomas include "Glioblastoma-IDH-wildtype", "Astrocytoma-IDH-mutant" and "Oligodendroglioma, IDH-mutant and 1p/19q-codeleted". While grading based on histologic features (grades 2-4) remains a part of the evaluation of IDH-mutant gliomas, the value of histologic grading in molecularly defined diffuse gliomas remains unclear. This study aims to evaluate the prognostic value of histologic grading in IDH-mutant astrocytomas and identify genomic alterations that may help predict overall survival (OS).
Methods: Astrocytoma, IDH-mutant cases [n = 186; UT MD Anderson Cancer Center (MDACC) and n = 56; UTHealth Science Center at Houston (UTH)] of grades 2-4 were included and demographic, clinical, radiologic, histologic, and sequencing data were obtained from the electronic medical records. Kaplan-Meier (log-rank) analysis was used to examine differences in OS between IDH-mutant astrocytomas of WHO grades 2-4.
Results: Including all patients (MDACC and UTHealth, n = 221), there was a significant difference in OS between IDH-mutant astroctyomas with grades 2, 3 and 4. No significant difference in OS was observed between astrocytomas with canonical vs. non-canonical IDH1/IDH2 mutations. Analysis of the correlation between OS and other genetic alterations is ongoing. Computer assisted quantitation of hotspot Ki67 index was significantly different between grades 2 (5.9%), 3 (14.0%) and 4 (25.7%).
Conclusions: These data support statistically significant differences in Ki67 labelling index and OS between grade 2, 3, and 4 IDH-mutant astrocytomas.

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Malignant Transformation of Low-Grade Glioneuronal Tumour into Diffuse Paediatric-Type High-Grade Glioma with Widespread Metastases

J Houpt[1], L Ang[1], C Hawkins[2], A Levine[2]
[1]London Health Sciences Centre Department of Pathology and Laboratory Medicine; [2]Hospital for Sick Children

Background: Diffuse paediatric-type high-grade glioma H3-wildtype and IDH-wildtype (PHGG) tumours are an aggressive subset of paediatric-type glioma with poor overall survival.
Methods: We report a case of a temporal lobe mass in a 4-year-old girl initially diagnosed as a ganglioglioma on gross

total resection, a local recurrence 11 years later reported as an anaplastic glioneuronal tumour (treated with resection and craniospinal radiotherapy), and diffusely metastatic disease over the following two years involving the bone marrow (biopsied from the left PSIS) and with a compressive mass impinging on the T6 spinal cord (resected).

Results: Histologically, the tumour contained areas of neurocytic and dysmorphic ganglion morphology with immunopositivity for neuronal markers (PGP9.5, NeuN). Ki-67 index varied from 8-35% and meningeal and brain invasion was observed. The metastases demonstrated similar histomorphology. Methylation array, DNA panel sequencing, and copy number analysis were performed on the resection specimens from the local recurrence and the epidural metastasis. This revealed a KIAA1549::BRAF fusion and a loss of 1p and gain of 1q in both tumours with no CDKN2A homozygous deletion. The metastatic lesion additionally carried a gain of function mutation in PIK3CA (H1047R) mutation and was identified using the DKFZ classifier as diffuse paediatric-type high-grade glioma H3-wildtype IDH-wildtype (0.85 confidence score). Interestingly, the locally recurrent tumour was only identified via NIH classifier with low confidence as a diffuse leptomeningeal glioneuronal tumour (DLGNT), an entity more characteristically associated with KIAA1549::BRAF fusion and 1p deletion.

Conclusions: While previous cases of DPHG with ganglionic differentiation have been reported, this represents the first known case arising from malignant transformation of a DLGNT with KIAA1549::BRAF fusion, acquired PIK3CA mutation, and diffusely metastatic disease. This is not only a rare case, but one which has provided us the opportunity to study the molecular basis of malignant transformation and metastasis in glioneuronal tumours.

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Glioblastoma-like outcomes in diffuse gliomas with proficient DNA damage response signature

Nalin Leelatian[1,2], Charu Singh[3], Richard Bouffard[2], Ranjini K. Sundaram[3], Juan C. Vasquez[5], Kirsten E. Diggins[4], Bret C. Mobley[1], Zeynep Erson-Omay[6], Ranjit S. Bindra[2,3]
[1]Department of Pathology, Microbiology, and Immunology, Vanderbilt University Medical Center, TN, USA; [2]Department of Pathology, Yale School of Medicine, CT, USA; [3]Department of Therapeutic Radiology, Yale School of Medicine, CT, USA; [4]Cyclo Technologies, GA, USA; [5]Department of Pediatrics, Yale School of Medicine, CT, USA; [6]Department of Neurosurgery, Yale School of Medicine, CT, USA

Background: Current treatment for adult-type diffuse gliomas exploits DNA damage response (DDR) pathways for therapeutic gain. However, clinical assessment of DDR integrity as a potential resistance mechanism is not routinely performed.

Methods: We characterized gene expression and DNA methylation profiles of 274 DDR genes of 593 TCGA gliomas. The pathologic diagnoses were assessed and re-assigned in accordance with the updated 2021 WHO classification prior to subsequent analyses. Single-cell quantitation of representative DDR pathway effectors by imaging mass cytometry was performed on a separate cohort of 118 gliomas to dissect intratumoral protein-level DDR heterogeneity

Results: Gene expression of 274 DDR genes revealed 4 distinct clusters (DDRr1-4) that corresponded to WHO 2021-defined histologic and molecular diagnoses and previously reported molecular subclasses. DDRr4 gliomas were associated with worse outcomes, regardless of their IDH mutation status. Specifically, DDRr4 IDH-mutant gliomas demonstrated similar clinical behaviors to IDH-wildtype glioblastoma. DDRr4-upregulated genes comprise enrichment of diverse DDR pathways, including base excision repair, nucleotide excision repair, mismatch repair, homologous end-joining repair, and Fanconi anemia. Methylation status of DDR gene transcription start sites showed similar association with pathologic diagnoses and molecular classes. However, methylation sites that epigenetically silenced gene expression did not, indicating alternative regulation mechanisms. Single-cell protein analysis revealed a subset of glioma cells with intact DDR effector expression. The abundance of DDR-proficient tumor cells was associated with worse survival, even among glioblastomas. In contrast, glioma cells with DDR protein downregulation portend a survival benefit and a subset of these cells demonstrated upregulation of CHK2 with aberrant cytosolic localization.

Conclusions: This study indicated that DDR landscape at both gene expression and protein levels have significant impact on the molecular classifications of diffuse gliomas. Tumors with proficient DDR signature carried poor clinical outcomes. Therefore, assessment of pre-therapy DDR footprints in gliomas may reveal cases that resist standard therapy, independent of other molecular signatures.

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High aneuploidy is a predictor of aggressive behavior in oligodendroglioma, IDH-mutant, 1p19q codeleted

S Belakhoua, J Serrano, M Garcia, C Schroff, I Tran, K Galbraith, M Snuderl
NYU Langone Health

Background: Oligodendroglioma, IDH-mutant, 1p19q codeleted are CNS gliomas that arise mostly in adults with a peak incidence in the fifth decade. CDKN2A/B homozygous deletion has been suggested as an indicator of poor prognosis and a biomarker of grade 3 according to CNS WHO classification of 2021.

Methods: We performed whole genome DNA methylation analysis using Illumina EPIC array of 82 tumors diagnosed by histology and 1p19q analysis as oligodendroglioma. DNA methylation data were analyzed using clinically validated brain tumor classifier, followed by unsupervised clustering analysis. The copy number profile was evaluated using conumee package. Mutational analysis was performed using FDA 510(k) cleared NYU Langone Genome PACT, a matched Tumor-Normal 607 exonic DNA NGS. Molecular results were compared with clinical, histological, immunohistochemistry data.

Results: Out of 82 samples, 76 classified as O_IDH using brain tumor classifier. The remaining tumors classified as astrocytoma IDH mutant (x4), low grade glioma MYB subtype (x1) or did not match with any category (x1). CDKN2A/B showed homozygous deletion in 4 cases, two of which classified as astrocytoma IDH mutant on methylation, and loss of heterozygosity (LOH) in 6 cases, five of which were graded CNS WHO 3 and one CNS WHO 2. Copy number analysis showed a complex pattern in 47 cases. Loss of heterozygosity (LOH) was detected for chromosomes 4 (n = 15), 14q (n = 13), 18 (n = 11), 15q (n = 10), 13q (n = 9) and 9 (n = 7). Nine cases showed gain of chromosome 7q. For the cases with chr4 LOH, 13 were considered grade 3 and only two were grade 2.
Conclusions: CDKN2A/B homozygous loss is extremely rare in oligodendroglioma. Aneuploidy is a more frequent molecular feature of aggressive oligodendroglioma. Therefore, genome wide assessment of aneuploidy may have a higher prognostic yield than targeted analysis of the CDKN2A/B locus.

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Tumor-Targeted pan-RAS Inhibition as a Novel Biologic Therapy for Diffuse Midline Glioma

C Hawkins, S Sbergio, P Liang, G Beilhartz, R Melnyk
The Hospital for Sick Children

Background: Diffuse Midline Glioma (DMG) has a poor outcome with limited successful treatment options beyond radiotherapy. Transcriptional characterization of these tumors shows almost universal upregulation of the RAS/MAPK pathway and as RAS regulates cellular proliferation and survival it makes a promising target. We developed a novel biologic based by creating a protein chimera using diphtheria toxin (DT) combined with a RAS cleaving peptidase (RRSP) and tested its efficacy in vitro and in vivo to treat DMG.
Methods: We treated SU-DIPG XVII, XXV, 36 and 50 cell lines with DT, RRSP, and RRSP-DTB constructs and measured viability using Cell Titer Glo Luminescent Cell Viability Assay. Western Blot was used to validate effective ablation of RAS following treatment. Proteomic analysis and single cell RNA sequencing was used to identify a cell surface receptor upregulated on tumor cell populations from patient samples. Using an orthotopic xenograft model, SU-DIPG 36 was injected intracranially and treated with RRSP constructs for 1 week via convection enhanced delivery and monitored for survival.
Results: RRSP-DTB reduced DIPG cell viability across a range of DMG patient derived lines with EC50 in the low picomolar range. By Western blot, RAS was ablated in RRSP-treated vs control cells. Single cell sequencing identified ANTXR1 receptor as upregulated in tumor cell populations vs normal brain cells, thus a TEM8 conjugated chimera was developed to specifically target tumor cells in vivo. RRSP-TEM8 chimera had an EC50 of ~ 40-100 pM in vitro and significantly improved survival in vivo with a median survival of 61 days compared to the vehicle control, 42 days ($p = 0.03$).
Conclusions: Our novel chimeric biologic, RRSP-TEM8, effectively targets DMG cells and reduces cell viability to improve survival in vivo representing a novel therapeutic approach for these deadly tumors.

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Clinical and molecular characterization of disseminated low-grade glioma

A Levine[1], C Li[1], J Haizel-Cobbina[2], L Nobre[1], J Bennett[1], M Dewan[2], U Tabori[1], C Hawkins[1]
[1]Hospital for Sick Children; [2]Vanderbilt University Medical Centre

Background: Although most pediatric LGG (PLGG) have excellent long-term survival, there is a subset of cases that disseminate throughout the neuraxis (DLGG) and have very poor outcomes. The reason for this aggressive behavior is unknown but we hypothesize that it has distinct and specific biological mechanisms. The methylation-based class of diffuse leptomeningeal glioneuronal tumor (DLGNT) is characterized by MAPK pathway activating fusions with 1p loss, 19q loss, and/or 1q gain. However, it is unknown what proportion of DLGG match the DLGNT group, and which other methylation classes are at risk of metastasis.
Methods: To improve our understanding of this rare patient population, we created an international DLGG consortium. In addition to detailed clinical annotations, tumor samples were profiled with targeted NGS sequencing and methylation arrays.
Results: Data from 68 cases shows a broad age distribution and no sex predilection. As expected, DLGG has much worse prognosis than the overall PLGG population. Virtually all DLGG progress at 5 years, compared to 25% of other PLGG, and DLGG are 5-times more likely to die at 10 years. We observe three patterns of dissemination – 35% present with a localized mass and have secondary dissemination, 50% with disseminated tumor and a dominant mass, and 15% with diffusely disseminated disease. In 47 patients with molecular testing, BRAF fusions accounted for 64% of driver alterations. Additional alterations were identified less frequently, including BRAF V600E (9%), FGFR1 alterations (9%), and KRAS mutations (4%). In 25 patients with methylation profiling, 10 (40%) confidently classified, illustrating that many cases do not fit a defined methylation group. The most common classification was pilocytic astrocytoma (5 patients), and surprisingly only two patients classified as DLGNT.
Conclusions: In sum this study illustrates the variable clinical behaviour associated with metastasis in PLGG and expands the range of molecular driver alterations, including ones previously unreported in DLGNT.

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Glioblastoma with FGFR3-TACC3 fusion and TERT promoter mutation: A case report and review of the literature

D Zenezan, Y Rong, I Akhtar, N Jhala, A Seth
Temple University Hospital

Background: Fibroblast growth factor receptor3 (FGFR3)-transforming acidic coiled-coil 3 (TACC3) gene fusion has been found in brain, lung, and head and neck cancers. Approximately 3% of gliomas have FGFR3-TACC3 fusion with characteristic histopathological and genetic features.
Methods: A 66-year-old male with a history of clear cell renal cell carcinoma, status post resection was found to have a 1.1 x 0.6 cm right frontal mass with patchy enhancement. Histologically the neoplastic cells from the resected mass were hypercellular spindle to ovoid infiltrating astrocytes, increased mitotic activity, and a network of thin capillaries. No necrosis or microvascular proliferation was noted. Molecular studies revealed FGFR3-TACC3 fusion and TERT promoter mutation without IDH mutations.
Results: FGFR3-TACC3 fusions are oncogenic drivers that have been reported in only 3% of IDH wild-type gliomas and some other cancers but have not been found in IDH mutant gliomas. Glioma cells with FGFR-TACC fusions, potent oncogenic events, are more sensitive to FGFR inhibitors.
Conclusions: IDH wild-type glioblastomas have a poor prognosis. Identifying patients with FGFR3-TACC3 fusion can potentially provide a targeted treatment.

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IDH2 (p.K155R) mutation in glioma

P Han
West Virginia University

Background: Among IDH mutant glioma, the most common mutation type is IDH1 (p.R132H).Other non-canonical IDH mutations include R132C, R132S, R132G, etc. IDH1 mutation appears to uniquely involve the specific site of R132, and the most frequent IDH2 mutation is R172K. Here we report a case with a rare IDH2 p.K155R mutation with unknown significance.
Methods: N/A
Results: Case description: The patient is a 50-year-old male who presented with left frontal enhancing mass and T2 FLAIR abnormality extending to the left thalamus, basal ganglia, and corpus callosum. Resection specimen showed hypercellular, diffusely infiltrating tumor with atypical elongated hyperchromatic nuclei. Mitotic figures are frequent. Microvascular proliferation and palisading necrosis are present. Immunohistochemically, stains show positive olig2, negative IDH1 (R132H), negative H3K27M, retained nuclear ATRX, wild-type pattern P53 and increased ki67 proliferation in the range of 20-30%. TERT promoter mutation (c. -146C>T), EGFR amplification, +7/-10 chromosome change are all present. MGMT is methylated. Whole exon sequencing revealed IDH2 (p.K155R) mutation that was interpreted as variants of uncertain significance.
Conclusions: Discussion: IDH2 mutation can be found in various high-grade cancer including Acute myeloid leukemia (AML), Glioma, Dedifferentiated chondrosarcoma (DDCHS), Angioimmunoblastic T cell lymphoma (AITL), Solid papillary carcinoma with reverse polarity (SPCRP), and Sinonasal undifferentiated carcinoma (SNUC). Invariably the mutations involve loss of arginine at the position of 172 or 140. Most mutation types are a substitute of arginine, a basophilic amino acid, by a neutral amino acid. Nevertheless, conversion to Lysine (R172K) has been found in AML, glioma, AITL. The significance of IDH2 (p.K155R) mutation, i.e. substitute lysine with arginine, remains unclear. It is also not sure if we should classify this case as IDH-mutant or IDH-wt glioma. It would be interesting to explore the significance of this mutation. As a matter of fact, this mutation had been shown to be associated with lysine's acylation in AML cell line.

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The Importance of Understanding the Histopathological Features of Tumor Inflammation-Associated Neurotoxicity

J Mahdi[1], J Dietrich[2], K Straathof[3], C Roddie[3], B Scott[1], T Davidson[4], L Prolo[1], T Batchelor[5], C Campen[1], K Davis[1], J Gust[6], M Lim[1], R Majzner[1], J Park[6], S Partap[1], S Ramakrishna[1], R Richards[1], L Schultz[1], N Vitanza[6], L Wang[7], H Vogel[1], C Mackall[1], M Monje[8]
[1]Stanford University; [2]Massachusetts General Hospital; [3]University College London; [4]University of Southern California; [5]Brigham and Women's Hospital and Harvard Medical School; [6]University of Washington; [7]City of Hope National Medical Center; [8]Stanford University, HHMI

Background: Immunotherapies represent promising treatment modalities for patients with central nervous system (CNS) tumors, and several clinical trials are now studying the use of therapies such as immune checkpoint inhibition, antibody-based therapies, adoptive cell therapies, including chimeric antigen receptor (CAR) T cell therapy, and oncolytic vaccines for the treatment of glioblastoma, H3K27M-altered diffuse midline glioma and primary CNS lymphoma. Immunotherapies exhibit unique toxicity profiles, distinct from more traditional, systemic cytotoxic therapies.
Methods: A localized neurotoxicity syndrome termed tumor inflammation-associated neurotoxicity (TIAN) has been observed in our collective experience of treating patients with immunotherapies for CNS tumors, which is distinct from the systemic inflammation seen with cytokine release syndrome and immune effector-cell associated neurotoxicity syndrome.
Results: TIAN arises secondary to localized inflammation at the tumor site causing local neuronal dysfunction and/or local inflammation-induced edema leading to tissue shifts and increased intracranial pressure (ICP). Encompassing the concept of "pseudoprogression," TIAN can be associated with treatment-induced increases in tumor size; however, it is important to note that TIAN can occur in the absence of edema, attributable to the powerful direct effects of inflammatory signaling molecules like cytokines and chemokines directly on neural cell function. There are two types of TIAN: 1) type 1 TIAN occurs when tumor inflammation-induced edema leads to mechanical space constraints and results in increased ICP, hydrocephalus; if unmanaged, may cause a herniation

syndrome 2) type 2 TIAN reflects inflammation-induced local neural electrophysiological dysfunction resulting in transient worsening/development of new neurological symptoms. The precise mechanisms underpinning the local neural dysfunction seen in TIAN remain to be understood and the histopathological biomarkers associated with TIAN have yet to be identified.
Conclusions: A greater understanding of histopathology associated with TIAN will not only help elucidate the neural mechanisms that drive TIAN but will also provide insight to the natural history of TIAN and inform clinical management.

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A case of glioneuronal tumor with BRAF mutation showing infiltrative growth pattern of glial cells

Y Nakayama[1], M Mizutani[2], T Sano[2], K Ijima[2], E Chiba[2], K Satomi[3], N Sato[2], M Iwasaki[2], J Shibahara[3], M Takao[2]
[1]Department of Pathology, Keio University School of Medicine; [2]National Center of Neurology and Psychiatry; [3]Kyorin University School of Medicine

Background: Polymorphous low-grade neuroepithelial tumor of the young (PLNTY), which was added to the fifth edition of the World Health Organization classification, is a tumor characterized by an abnormal mitogen-activated protein kinase (MAPK) pathway and diffuse proliferation of oligodendroglioma-like cells. Although not typical, small amounts of dysmorphic neurons may also be present. Ganglioglioma is a tumor composed of ganglion cells and glial cells that may also have an abnormal MAPK pathway and often presents as a microscopic infiltrative growth.
Methods: We herein present a case involving a 17-year-old female patient who was diagnosed with focal epilepsy at the age of 15 years. Magnetic resonance imaging findings showed a multi-cystic, ill-defined mass in the right medial temporal area with calcifications and focal enhancement. She was referred to our epilepsy center at the age of 17 years because her seizures were refractory to anti-epileptic drugs. She then underwent surgical removal of the anterior part of the right temporal lobe including the tumor, hippocampus, and parahippocampal gyrus.
Results: Histopathologic examination revealed infiltrative proliferation of glial cells in the white to gray matter. The neoplastic glial cells included oligodendroglia-like cells, and the cellular atypia was mild. In the hippocampus, similar neoplastic glial cells and a cluster of dysmorphic neurons were observed. Partial calcification was also present. Immunohistochemistry showed CD34-positive cells and BRAFV600E-positive cells. Pathological and molecular pathological findings suggested a glioneuronal tumor, which required differentiation from PLNTY and ganglioglioma.
Conclusions: PLNTY and ganglioglioma partly overlap both molecularly and histopathologically and may be difficult to distinguish in some cases. We will add DNA methylation analysis and re-examine the tumor type, and we will present the results on the day of the meeting.

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Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype, with MYCN amplification mimicking an embryonal tumor

J Berry[1], B Orr[2], H Harmsen[1]
[1]Vanderbilt University Medical Center; [2]St. Jude Children's Research Hospital

Background: Diffuse pediatric-type high grade glioma, H3-wildtype and IDH-wildtype, can be subdivided into the RTK1, RTK2, and MYCN subtypes by DNA methylation profiling. While preferentially arising in the supratentorial compartment of children, they are occasionally found in the posterior fossa where the clinical presentation can mimic that of the more common diffuse midline glioma, H3K27-altered. The histologic features range from those of an infiltrating high-grade glioma to those of an embryonal tumor. The prognosis of these lesions is overwhelmingly poor, warranting a CNS WHO grade 4 designation.
Methods: We present the case of a 3-year-old boy who presented with several days of coordination and gait changes. Magnetic resonance imaging (MRI) revealed a 3.5-cm enhancing, circumscribed mass centered in the midbrain and involving the left diencephalon and pons with associated T2 signal abnormalities in the pons and cerebellum.
Results: An open biopsy revealed sheets of densely packed, highly proliferative cells with enlarged, hyperchromatic, ovoid-to-angular nuclei and scant cytoplasm within a neuropil-like stroma. The tumor cells, which expressed diffuse Olig2 and SOX2 and patchy synaptophysin, were largely negative for GFAP and demonstrated non-mutant staining patterns for H3K27M, INI1, and BRG1. Molecular testing detected MYCN amplification, and methylation subtyping confirmed the diagnosis of "Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype, MYCN subtype."
Conclusions: Tumors within the methylation class "Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype, MYCN subtype" represent a highly aggressive class of pediatric high-grade gliomas which can present a diagnostic challenge. Unlike other diffuse gliomas, this subtype can be markedly circumscribed both radiologically and histologically. The morphologic features can resemble those of embryonal tumors with mitotically active undifferentiated tumor cells that may variably express glial and neuronal markers. A broad immunohistochemical evaluation with multiple glial markers and mutation-specific stains, in addition to diagnostic molecular testing, may be required to accurately classify these lesions.

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Unclassified Intraventricular Low-grade Glioneuronal Tumor with Abundant Astrocytic Melanin Pigment and Focal Piloid Feature

H Miyata[1], S Kimura[2], M Moritsubo[3], T Furuta[3], Y Sugita[4], S Kono[2], S Fushimi[2]

[1]Department of Neuropathology, Research Institute for Brain and Blood Vessels, Akita Cerebrospinal and Cardiovascular Center; [2]Japanese Red Cross Society Himeji Hospital; [3]Kurume University School of Medicine; [4]St. Mary's Hospital

Background: Melanin production of unknown pathogenesis and pathological significance has rarely been described in neuroepithelial tumors, including choroid plexus papilloma, ependymoma, gliomas, ganglioglioma, and central neurocytoma.
Methods: A 34-year-old, previously healthy Japanese female presented with a headache lasting for a month. Brain magnetic resonance imaging revealed a mass lesion with cystic changes occupying the third and left lateral ventricles and obstructive hydrocephalus. The patient underwent surgical removal of the tumor; however, she suffered a tumor regrowth two months later. She underwent reoperation for tumor resection and ventricular drainage at another hospital, followed by intensity-modulated radiation therapy and chemotherapy.
Results: Microscopically, the tumor from the initial surgery consisted mainly of neoplastic glial cells (GFAP-, olig2+, MAP2+) having scant cytoplasm and a few short processes proliferating diffusely or along the hemosiderin-containing microcystic walls and melanin-containing astrocytic tumor cells (GFAP+, Melan A-, HMB45-), with basophilic myxoid degeneration and hyalinized vessels. There was a focal pilocytic astrocytoma-like area (GFAP+, olig2-, MAP2-) containing scattered eosinophilic granular bodies and calcification, but Rosenthal fibers were not observed. A few large atypical nuclei and mitotic figures were present with a Ki-67 labeling index of 4.8%; neither necrosis nor microvascular proliferation was observed. The tumor also contained an unequivocal cluster of small to medium-sized immature neurons (cytoplasmic synaptophysin+, neurofilament+, NeuN±, olig2±, HuC/D+) with a high nuclear-to-cytoplasmic ratio and occasional binucleation. There were no neurocytic rosette, neuropil-like components, specific glioneuronal elements, or ependymal differentiation. Genetic analysis using paraffin sections revealed FGFR1 p.K656E mutation. FGFR1 p.N546, BRAF p.V600, IDH1, and IDH2 were wild types.
Conclusions: Astrocytic components of central neuroepithelial cell derivatives and neoplastic astrocytes are known to be capable of melanosomal melanin production. Continuous tumor exposure to cerebrospinal fluid may have contributed to the degenerative changes and abundant astrocytic melanin pigment in this difficult-to-classify low-grade glioneuronal tumor with focal piloid feature.

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A Diffuse Glioma with IDH-mutation, ATRX-loss, and 1p/19q-codeletion: A Case Report

L Endelman[1], M Salamat[1], M Baskaya[1], R Jenkins[2], K Aldape[3], J Helgager[1]
[1]University of Wisconsin Hospitals and Clinics; [2]Mayo Clinic; [3]National Cancer Institute

Background: Inactivating mutations in ATRX, as first suggested by cIMPACT-NOW update 2 and subsequently codified in the 5th edition of the WHO Classification of Tumors of the Central Nervous System, are diagnostic of astrocytoma, IDH-mutant, in the context of an IDH-mutant diffuse glioma. Furthermore, they are thought to be incompatible with oligodendroglioma biology, which almost exclusively harbor TERT promoter activating mutations, and ATRX loss by immunohistochemistry obviates the need for subsequent 1p/19q-codeletion testing.
Methods: We present a case of a 61-year-old man who presented with a diffuse glial neoplasm with histology suggestive of oligodendroglioma.
Results: Immunohistochemical and molecular work-up demonstrated mutations in IDH2 and ATRX, the latter resulting in immunohistochemical ATRX loss, absence of a TERT promoter mutation, 1p/19q co-deletion, and mutations in CIC and PPM1D. Subsequent methylation-based tumor profiling matched a consensus methylation profile class of "IDH glioma, subclass 1p/19q-codeleted oligodendroglioma".
Conclusions: Though examples of such tumors with hybrid oligodendroglioma and astrocytoma molecular features have rarely been reported, this is the first case to our knowledge that has been profiled by DNA methylation, and when taken together with the histology and other supportive molecular alterations seems most compatible with the diagnosis of oligodendroglioma. The implications of such exceptional neoplasms with respect to the present guidelines for tumor classification and work-up deserves further consideration, along with the possibility that oligodendrogliomas may very rarely utilize the alternative lengthening of telomeres mechanism as a means of maintaining telomere length, as opposed to the canonical process of telomerase activation.

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A Rare Case of Primary Peritoneal Ependymoma in a young woman

S Haddad, E Borys, D Borys
Loyola University Medical Center

Background: Ependymomas rarely occur outside of the central nervous system and have previously been reported in unusual anatomical locations, such as the ovary, para-ovarian tissues, pelvis, paracervical soft tissue, lung, omentum, small bowel, and posterior mediastinum. Primary peritoneal ependymomas are exceedingly rare, with only five cases found in the literature.
Methods: We describe a case of a 19-year-old otherwise healthy woman presenting with abdominal pain, bilateral pleural effusions, and ascites. Imaging demonstrated a 19.8 cm intraabdominal and intrapelvic mass with extensive solid nodularity of the peritoneum along the anterior and posterior cul de sac and pelvic sidewalls. The mass involved the diaphragm, sigmoid, rectum, and omentum and adhered to the left adnexa. Blood biochemistry showed elevated CA125 levels (795 U/ml; normal, 0-35 U/ml). The patient underwent exploratory lapa-

rotomy, tumor debulking with bilateral salpingo-oophorectomy, and infra-colic omentectomy.
Results: Microscopic examination showed the proliferation of monomorphic cells with round to oval nuclei with speckled chromatin and indistinct cytoplasmic borders. Well-formed perivascular pseudorosettes, true ependymal rosettes and canals were identified. There was no significant mitotic activity, microvascular proliferation, or necrosis. Immunohistochemical staining for glial fibrillary acidic protein (GFAP) and S100 highlighted the perivascular pseudorosettes and were variably positive in the cytoplasm of the tumor cells. A perinuclear dot-like staining pattern with epithelial membrane antigen (EMA) was also seen. Pathologic findings were consistent with an ependymoma. No pre-existing teratoma was identified. Subsequent central nervous system imaging studies were negative.
Conclusions: This case highlights the need to consider ependymoma as a potential differential diagnosis in ovarian or peritoneal malignancy in order to provide appropriate clinical management. Despite benign morphology, these tumors may behave aggressively.

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A Case Involving a Pleomorphic Xanthoastrocytoma Highlighting the Morphological Heterogeneity of These Eccentric Entities

L Robinson[1], E Goold[2], Q Mao[2], S Potter[2]
[1]University of Utah Department of Pathology; [2]University of Utah Pathology Department

Background: Herein, we present a case of a 43-year-old female who presented with a yearlong history of progressive neurological complaints involving numbness and pain in the bilateral lower extremities (right > left) and hands. Magnetic resonance imaging of the brain showed a well-circumscribed, enhancing, cortically-based mass (1.0 x 0.9 cm) in the left parietal lobe. Histopathological examination of the right parietal mass demonstrated a well-circumscribed, hypercellular glioma composed of cells with astrocytic features involving oval-to-elongated, hyperchromatic tumor cell nuclei arranged in uniform lobules. The mitotic index was 1/10 high-power-fields, and no necrosis or microvascular proliferation was identified. Immunohistochemical profiling of the tumor cells revealed: positive GFAP, negative IDH1-R132H, focally weak positive synaptophysin, chromogranin-A, and CD34, and no infiltrating axons around tumor cells highlighted by neurofilament. Overall, the initial diagnostic impression was a circumscribed astrocytic glioma, CNS WHO Grade 2.
Methods: To further classify the tumor, molecular testing was ordered.
Results: The BRAF pyrosequencing detected the p.V600E variant. DNA methylation-based profiling further classified the tumor as having a CDKN2A deep deletion with a final diagnosis of a pleomorphic xanthoastrocytoma (PXA), CNS WHO Grade 2.
Conclusions: PXAs are generally low-grade, astrocytic tumors primarily occurring in children and young adults that characteristically harbor BRAF V600E mutations and homozygous CDKN2A/CDKN2B deletions. Radiologically, these tumors are peripherally located involving the leptomeninges and temporal lobes while often possessing a cystic component. Histologically, PXAs have been characterized by demonstrating a mixture of large pleomorphic (frequently multinucleated) cells, lipidized cells, and spindle cells with associated eosinophilic granular bodies. The staining profile for PXAs typically show positive expression for GFAP and CD34 with abundant reticulin deposition. This unique case further highlights the importance of molecular profiling regarding PXAs and how they can exhibit a broader, heterogeneous morphological spectrum than the original histological description of this tumor type.

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Long Survival of Supratentorial Neuroepithelial Tumor with PLAGL1 Fusion: A Case Report

O Ogunbona[1], M McDonald[2], C Helfer[2], K Aldape[3], Z Abdullaev[3], J Mandel[2], H Lu[2]
[1]Houston Methodist; [2]Baylor College of Medicine; [3]National Cancer Institute (NCI)/National Institutes of Health (NIH)

Background: Recently, a novel class of rare supratentorial pediatric-type tumors with ependymoma-like histology and unique methylome signature has been described. The tumor has recurrent fusion involving PLAGL1, with EWSR1 or FOXO1 as the fusion partner. The limited cases reported have a median progression-free survival of 35 months. Here, we describe a case with 45 years of survival.
Methods: The electronic medical record was used to obtain the clinical history and radiologic findings. A histopathological examination was carried out, as well as targeted next-generation sequencing and DNA-methylation profiling.
Results: The patient initially presented with vomiting at the age of 2 and was discovered to have a grapefruit-sized left frontal mass. The tumor was resected and diagnosed as a grade 1 subependymoma. He later developed epilepsy at the age of 9. His seizure has become more frequent in recent years. A follow-up MRI showed a left frontal lobe encephalomalacia with nodular cortical and/or dural thickening and enhancement in the same region, and he underwent re-resection. Histologically, the tumor was moderately cellular with numerous perivascular pseudorosettes. No significant mitoses, vascular proliferation, or necrosis were present. The tumor cells were strongly positive for GFAP with a Ki-67 proliferative index of < 2%. EMA, IDH1 (p.R132H), and BRAF (p.V600E) immunohistochemical stains were negative. Targeted sequencing showed no mutations in IDH1/2. Methylation profiling showed that the tumor clusters with the newly described supratentorial neuroepithelial tumor with PLAGL1 fusion, and PLAGL1-FOXO1 fusion was confirmed by RNA sequencing.
Conclusions: Despite having a distinctive methylome signature, it is not yet apparent if the supratentorial neuroepithelial tumor with PLAGL1 fusion should be categorized as a subtype of ependymal neoplasm or as a completely independent entity. Compared to cases that have been previously documented, this case has a significantly less aggressive course. Additional cases

will help clarify the clinicopathological features and taxonomy of this entity.

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Detailed genetic assessment of an oligosarcoma with retained IDH-mutation and 1p/19q codeletion at time of transformation

A Ziaei[1], M Terry[2], C Stoos[3], A Perry[1]
[1]Department of Pathology, University of California, San Francisco (UCSF), Division of neuropathology; [2]University of California, San Francisco (UCSF); [3]Methodist Health System. 8303 Dodge Street, Omaha, NE 68114

Background: Oligodendroglioma with sarcomatous transformation ("oligosarcoma" or "oligodendroglial gliosarcoma") is a rare form of IDH-mutant glioma.
Methods: The authors present a case of oligosarcoma arising in a 42-year-old man with a history of left frontal oligodendroglioma, 1p/19q co-deleted, CNS WHO grade 2.
Results: Eight years after completing a course of radiation therapy to his brain, a follow-up brain MRI showed increasing rim enhancement in the prior resection cavity, suggesting tumor recurrence vs radiation necrosis. Resection of the enhancing mass revealed an infiltrating and solid glial neoplasm associated with a dense fibrous stroma. Focally, tumor cells had an oligodendroglioma-like cytologic appearance, along with microvascular proliferation. In most areas, tumor cells had a sarcomatous appearance, with spindled, pleomorphic, enlarged, and hyperchromatic cells with foci of necrosis and high mitotic index, suggestive of sarcomatous transformation. UCSF500 next-generation sequencing analysis was performed separately of the oligodendroglioma and the sarcomatous components. Both components exhibited whole arm 1p/19q co-deletion, as well as IDH1, TERTp, FUBP1, ARID1A, and NOTCH2 mutations. The oligodendroglioma component additionally showed a NOTCH1 mutation, and the sarcoma component showed additional copy number changes and CDKN2A deletion. DNA methylation profiling of both components is being performed, and the results are pending.
Conclusions: Oligosarcomas are rare and portend a poor prognosis. They are at least CNS WHO grade 3 if considered an oligodendroglioma variant versus a CNS WHO grade 4 if considered a gliosarcoma variant.

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A combined cyclin D1, SOX10, and p16 immunopanel is specific in distinguishing MAP kinase activated gliomas from reactive gliosis

V Tang[1], K Mirchia[1], R Lu[1], E Guney[1], M Terry[1], C Lucas[2], A Perry[1]
[1]University of California, San Francisco; [2]Johns Hopkins University

Background: Pilocytic astrocytoma (PA) and ganglioglioma (GG) represent the most common pediatric low-grade gliomas (LGGs). Yet, their histopathologic features can be challenging to distinguish from reactive gliosis, particularly in a small or limited biopsy. PA and GG are associated with alterations that activate the mitogen-activated protein kinase (MAPK) pathway, with cyclin D1 being one downstream regulator that may be useful in identifying pathway activation. Additionally, SOX10 and p16 are widely expressed in PAs and other LGGs. We therefore aimed to investigate whether these markers can reliably distinguish LGGs from reactive gliosis.
Methods: We performed cyclin D1, SOX10, and p16 immunohistochemistry (IHC) on a cohort of 30 samples of MAPK-driven neoplasms (PA and GG) versus reactive astrogliosis (including those in reactive gliosis alone, acute inflammatory demyelinating disease, and other histiocyte-rich lesions). Protein overexpression was defined as greater than 50% staining among glial cells.
Results: Of 15 neoplastic cases, protein overexpression was found in 5 (33%) for cyclin D1, 11 (73%) for SOX10, and 12 (80%) for p16. In contrast, of 15 gliotic cases, overexpression was seen in 2 cases each (13%) for cyclin D1, SOX10, and p16. The specificity of each individual marker was 87% (13/15). Overexpression of at least two markers had a 100% positive predictive value and 100% specificity (10/10 and 15/15, respectively) in distinguishing neoplasm from gliosis, with a sensitivity of 67% (10/15) and negative predictive value of 75% (15/20).
Conclusions: Although cyclin D1, SOX10, and p16 are variably sensitive and specific, expression of at least two of these three markers increases specificity in distinguishing MAPK-driven LGGs from gliosis. These results support a potential use of these IHC markers as an accessible and cost-effective diagnostic aid in identifying histologically challenging LGGs.

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Neuroepithelial tumor with PATZ1 fusion: 27 years of clinical follow-up

C Xing[1], K Fink[2], B Mickey[1], J Raisanen[1], K Hatanpaa[1]
[1]UTSW; [2]Baylor Scott & White Neuro-Oncology Associates

Background: PATZ1 fusions have been described in glial tumors and extracranial sarcomas. The histological spectrum is broad and includes tumors resembling glioblastomas and astroblastomas; however, tumors with PATZ1 fusions form a distinct cluster by methylation profiling.
Methods: We present a 34-year-old female who was found to have an 8 mm focus of enhancement in the left parietal lobe. Over the next 8 years, the lesion grew to a 5 cm, uniformly enhancing, well demarcated mass. She developed difficulty typing with her right hand as well as partial seizures. She underwent a gross total resection in 2004.
Results: The tumor was difficult to classify but showed ependymoma-like features including epithelioid cells, perivascular rosettes, hyalinized vessels, compact architecture, and absence of significant infiltration. She received radiation for a recurrence in 2006. In 2016, she underwent a resection for a new, 1 cm focus of enhancement adjacent to the resection cav-

ity. The pathology was similar to the previous specimen. NGS revealed an EWSR1-PATZ fusion. She currently has no evidence of recurrence. She has mild neurological deficits due to radiation changes in the white matter of her left parietal lobe. The follow-up described here is the longest that has been documented for a glioma with a PATZ1 fusion.
Conclusions: Our findings illustrate the potential for a long survival with careful monitoring and multidisciplinary treatment approach. When the tumor is histologically noninfiltrating, the pathologist has a key role in communicating this finding and advocating for a gross total resection, even at a time of a recurrence.

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Pediatric brain tumor with features highly suggestive of DGONC and methylation study defined as adult-type high grade glioma

B Maveal[1], G Friedman[2], J Johnston[2], L Rong[3]
[1]University of Alabama Birmingham; [2]University of Alabama; [3]Children's Alabama

Background: The patient is an 8-year-old female with a history of developmental delay and epilepsy who presented with right facial droop. MRI revealed a large heterogeneous frontal temporal brain tumor involving the left insula, infundibulum, and left optic structures with midline extension into the suprasellar cistern and encasement of the internal carotid arteries and major branches of the circle of Willis. The left frontal craniotomy specimen revealed a moderately hypercellular diffusely infiltrating glioneuronal tumor. A large subset of the tumor cells are relatively uniform with round nuclei and perinuclear halos in conjunction with clusters of large tumor cells with hyperchromatic nuclei. There are areas of calcification, foci of necrosis, and scattered mitotic figures.
Methods: IHC, NGS, Methylation
Results: The tumor cells are positive for OLIG2, SOX10, and synaptophysin with rare positivity for NeuN and Neurofilament. The tumor cells are negative for GFAP and TTF-1. Ki-67/MIB reaches as high as 30%. Next generation sequencing study at the University of California San Francisco (UCSF) revealed an inactivating missense mutation in DNTMT3A and chromosomal alterations involving gains of 1q, distal 3q, 17q, 21q and losses of 1p, distal 3p, 6q, 13q, 14q, and 17p. Initial methylation study using DKFZ CNS Classifier v11b6 at Nationwide Children's Hospital showed no match but suggestive of 'CNS neuroblastoma with FOXR2 activation", which is usually clustered with DGONC. Subsequent methylation microarray analysis using version 12.6 of the Heidelberg classifier at the National Institutes of Health/National Cancer Institute (NIH/NCS) reported a high confidence score with an Adult-type diffuse high-grade glioma, IDH-wildtype, subtype E profile.
Conclusions: This case illustrates the need for further study to best characterize rare glioneuronal tumors and the integration of ancillary testing findings for diagnosis, prognosis, and treatment options.

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Impact of subclonal IDH mutation status on overall survival in high-grade gliomas

M Vij[1], W Cobb[2], O Rashidipour[3], T Richardson[3], M Snuderl[4], N Tsankova[3], J Walker[3], R Yokoda[3], R Yong[3]
[1]Mount Sinai Hospital; [2]Valley Hospital; [3]MSH; [4]NYUMC

Background: IDH mutations are thought to represent an early oncogenic event during glioma evolution, found with high penetrance across tumor cells. We present two unique cases with subclonal IDH1 R132H mutation and validate this rare phenomenon in a large cohort of IDH-mutant astrocytomas, wherein we establish its significant association with lower overall survival in high-grade tumors.
Methods: Case 1 is a 68-year-old female with a de-novo histologically grade 4 glioma. Immunohistochemical (IHC) studies revealed diffuse TP53 positivity, intact nuclear ATRX, and rare IDH1 R132H positivity in $< 1\%$ of cells. Next-generation sequencing (NGS) confirmed subclonal IDH1 R132H with variant allele frequently (VAF) of 0.7%; TP53 R282W (89.7% VAF); and wildtype ATRX. Interestingly, high tumor mutational burden (14 Muts/Mb TMB) was detected, along with a MSH2 splice site mutation (86.7% VAF) and several subclonal mutations, raising the possibility of mismatch repair deficiency. While the de-novo presentation of a glioma in this older patient and the subclonal nature of IDH1 R132H in the context of high TMB favored IDH-wildtype glioblastoma, DNA methylation classified the tumor as high-grade IDH-mutant astrocytoma with high confidence (0.98 score). Case 2 is a 42-year-old female with a recurrent/progressive grade 4 IDH-mutant astrocytoma. IHC detected IDH1 R132H in a minority of cells. NGS confirmed IDH1 R132H (11.7% VAF), TP53 I255N (69.2% VAF), and mutant ATRX (22.4% VAF). Two large publicly-available cohorts were mined for subclonal IDH1 mutations (defined as IDH1:TP53 mutation ratio < 0.33) and impact on overall patient survival.
Results: Subclonal IDH1 R132H was identified in 3.9% (18 of 466) IDH-mutant astrocytomas and had a significantly worse overall survival ($p = 0.0158$), in grade 3 and 4 tumors only. No significant association was seen with CDKN2A homozygous deletion and MGMT methylation status.
Conclusions: Our findings highlight the potential clinical utility of quantified IDH1 R132H expression by IHC and/or VAF by NGS.

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Supratentorial non-RELA, ZFTA-fused Ependymoma – A Characteristic Fusion with a Histopathologic Correlate

B Ramlal[1], F Rodriguez[2], V Paulson[3], K Aldape[4], M Morris[5]
[1]Johns Hopkins University School of Medicine; [2]Ronald Reagan UCLA Medical Center; [3]University of Washington Medical Center; [4]National Cancer Institute Center for Cancer Research; [5]Johns Hopkins Pathology

Background: The fifth edition of the WHO Classification of Tumors of the Central Nervous System incorporated novel diagnostic techniques, such as DNA methylation profiling, which resulted in the classification of new tumor classes and subtypes in order to establish a unified classification system. Ependymal tumors are classified based on a combination of histopathologic and molecular features, and are further divided based on their anatomic compartments; supratentorial (ST), posterior fossa (PF), and spinal (SP). ST ependymomas consist of two distinct molecular subtypes that are dominated by fusions involving either YAP1 or ZFTA. ZFTA-fusion ependymomas account for the majority of supratentorial ependymomas and are common in children. The most common fusion partner in this tumor is RELA; however, fusion partners can also include NCOA1/2, MAML2/3, or CTNNA2. These tumors have a morphologic spectrum that range from classic ependymal morphology to tumors with morphology similar to astroblastomas, pleomorphic xanthoastrocytoma, or high-grade diffuse gliomas. ZFTA::NCOA fusion ependymomas in particular can show areas of embryonal histology and sarcomatous differentiation.
Methods: Here we report the case of a 4 year-old female with a large solid and cystic mass involving the right parieto-occipital lobe. The case was evaluated using standard surgical pathology methods, targeted NGS, fusion testing, and methylation profiling.
Results: On histology, the tumor had predominantly embryonal morphology with areas of osteosarcomatous differentiation. NGS studies were negative for tumor-specific molecular drivers, and no copy number variants were identified. Fusion studies were negative for a RELA fusion. Subsequent evaluation of the patient's recurrence using a DNA-based targeted NGS panel at the University of Washington (UW OncoPlex) detected a ZFTA::NCOA2 fusion, prompting methylation reanalysis (NIH) using the recently released v12b6 DKFZ classifier, which matched to "Supratentorial Ependymoma, ZFTA fusion-positive".
Conclusions: While this case is unusual in the lack of traditional ependymal morphology, the morphologic findings are consistent with the underlying ZFTA::NCOA2 fusion.

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Multicenter observational study of adverse effects following GammaTile implantation 30 days post operatively in Intracranial Brain Neoplasms

F Davis[1], S Lee[2], S Peach[1], T Lodhi[3], D Brachman[4], STaRT Registry Investigators[4], P Boyer[1]
[1]ECU Health Medical Center; [2]ECU Health Neurosurgery; [3]Brody School of Medicine, East Carolina University; [4]GT Medical Technologies

Background: Placement of a radiation source within a neoplasm, brachytherapy, has been used effectively to treat a variety of neoplasms and avoid external beam radiation and associated morbidities. The Food and Drug Administration recently cleared the use of an implanted device containing cesium-131 seeds embedded in a collagen carrier tile, GammaTiles (GT Medical Technologies, Tempe, AZ), for use after resection in newly diagnosed malignant and recurrent intracranial neoplasms.
Methods: This study summarizes and reports findings from multiple centers deploying GammaTiles as part of treatment of brain neoplasms. The objective of the study was to (1) capture demographics, histopathology, survival, local control, adverse events (AE), and quality of life and (2) evaluate the patterns of clinical application and safety profiling through characterization of morbidity, mortality, and readmission within 30 days across institutions and tumor types.
Results: Through October 11, 2022, 72 patients from 16 enrolling institutions had completed the 30-day postoperative evaluation. Data was abstracted from the study registry on patients with possible, probable, or definitively attributable surgical- or radiation-related grade $\geq$3 AE. Of 72 patients (29 glioblastomas, 22 metastatic neoplasms, 8 meningiomas, 12 other primary tumors), 66 (83%) had recurrent/progressive after prior therapy. An attributed Grade $\geq$3 AE was noted in 7 cases (8.8%) including cerebral edema, intracranial hemorrhage, new onset hemiparesis, transient expressive aphasia, pseudomeningocele, and new onset seizure disorder; there were no wound infections.
Conclusions: GammaTile therapy is emerging as an important treatment option for brain neoplasms. The 30-day morbidity and readmission rates appear similar to those previously reported for patients undergoing conventional craniotomy for resection of a neoplasm and support a highly-favorable safety profile for this therapeutic approach. Follow-up and accrual are ongoing and future reports will help benchmark clinical outcomes of this treatment, allow comparison to existing treatments, and facilitate future clinical trials.

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Dual genotype oligoastrocytoma: Persistence of a tentative entity

E Medina-Parrilla, M Rivera-Zengotita
University of Florida

Background: Oligoastrocytoma was recognized as a distinct entity in previous editions of the WHO Classification of CNS Tumors and it was defined as a diffusely infiltrating glioma demonstrating neoplastic cells with both oligodendroglial and astrocytic morphology. In recent years, advances in molecular diagnostics allowed reclassification of most "oligoastrocytomas" to more objectively defined glioma types. As a result, the most recent edition of the CNS WHO Blue Book (WHO CNS5) removed oligoastrocytoma as a distinct tumor type but allows the tentative designation of "dual genotype oligoastrocytoma NEC".
Methods: We report a 36-year-old man with no significant past medical history that presented with recent onset headache, nausea, vomiting and syncope. Brain MRI demonstrated an intra-axial, 6.2 x 3.4 cm, right frontal lobe mass that showed heterogeneous contrast enhancement. The patient underwent a craniotomy for resection of the tumor.
Results: Histologic examination of permanent sections showed a cellular diffuse glioma with significant mitotic activity

and microvascular proliferation. Most of the tumor consisted of cells with non-uniform, hyperchromatic nuclei, including multinucleated forms, resembling astrocytoma (region A). A small but well-defined focus of tumor showed round, uniform nuclei with perinuclear haloes resembling oligodendroglioma (region B). Both regions were immunoreactive for IDH1 (R132H). Molecular testing revealed the following profile in each region: Region A: IDH1 R132H, TP53, and ATRX alterations by NGS; no 1p/19q codeletion by FISH. Region B: IDH1 R132H and TERT alterations by NGS; 1p/19q codeletion by FISH.

Conclusions: In conclusion, this IDH-mutant diffuse glioma showed two morphologically distinct cell populations and a dual oligoastrocytoma genotype. This case illustrates the morphological and molecular heterogeneity of diffuse gliomas. Although exceedingly rare, oligoastrocytomas with dual genotype deserve further study to determine prognostic and therapeutic implications of genetically "mixed" gliomas.

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Diffuse Hemispheric Glioma with H3-3B G34R Mutation

A Nazem[1], J Lavezo[2], Z Abdullaev[3], K Aldape[3], M Quezado[3], P Cimino[4], R Jenkins[1], C Ida[1]
[1]Mayo Clinic; [2]Texas Tech University Health Sciences Center of El Paso; [3]National Cancer Institute, National Institutes of Health; [4]National Institute of Neurological Disorders and Stroke, National Institutes of Health

Background: Diffuse hemispheric glioma, H3 G34-mutant (DHG_H3G34) is a new 2021 CNS WHO entity defined as a hemispheric cellular infiltrative glioma with mitotic activity that harbors an H3.3 p.G35R (G34R) or p.G35V (G34V) mutation (involving H3-3A gene), and for unresolved lesions, with a methylation profile matching DHG_H3G34; OLIG2 immunonegativity, loss of ATRX expression and diffuse p53 immunopositivity are diagnostic supportive findings. H3-3A and H3-3B genes (previously H3F3A and H3F3B) reside in different chromosomes and have distinct sequences but encode identical replication-independent histone H3.3 proteins.

Methods: We describe a tumor that fulfills all diagnostic criteria for DHG_H3G34 and harbors an H3-3B, instead of H3-3A, p.G35R (G34R) mutation. A 47-year-old male without significant past medical/family history presented with a four-week history of progressive cognitive decline, gait instability and speaking difficulty. MRI showed extensive right frontal non-enhancing T1 hypointensity/T2 hyperintensity within deep white matter extending into left frontal lobe.

Results: A biopsy was performed and showed a mitotically-active cellular infiltrating glioma. Tumor cells had scant cytoplasm with irregularly shaped hyperchromatic and occasionally pleomorphic nuclei, and were immunohistochemically negative for OLIG2, ATRX and IDH1-R132H, and diffusely positive for p53. Initial 118-gene targeted NGS testing including H3-3A, but not H3-3B gene, detected TP53x2, ATRX and PTPRD clinically relevant mutations. Methylation array profiling matched to DHG_H3G34 with high confidence score in DKFZ v.11.5/v.12.6 and NCI-EPIC classifiers and predicted methylated MGMT promoter. Interestingly, follow-up TruSight oncology 500 NGS panel testing detected an additional H3-3B c.103G>A p.G35R (G34R) mutation. Chromosomal microarray revealed a complex copy-number pattern including changes recurrently reported in DHG_H3G34 such as 3q, 9p and 13q losses.

Conclusions: To our knowledge this is the first report of a molecularly, cytogenetically, and epigenetically well-characterized DHG_H3G34 harboring an H3-3B, instead of H3-3A, G34 mutation, supporting the inclusion of H3-3B in the spectrum of histone H3 genes associated with this tumor type.

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57 yo man with high grade astrocytoma with piloid features and 2 other CNS lesions

J Fullmer
Corewell Beaumont University Hospital

Patient is a 57 yo man who presented with periodic headaches and lower back pain, initially attributed to lifting heavy weights but symptoms persisted. Eventual imaging was performed and revealed a 4 cm heterogeneously enhancing left cerebellar mass, 1 cm enhancing lateral right frontal subcortical lesion, and enhancing T12-L1 spinal cord lesion and multiple smaller spinal cord lesions suggestive of drop metastasis. The cerebellar lesion was first resected and final results, including methylome profiling, were consistent with high grade astrocytoma with piloid features (HGAP). The T12-L1 spinal lesion was later resected and had somewhat distinct histology although intraoperatively surgeon felt it appeared similar but was also sent off for methylome profiling, the results of which are currently pending. The subcortical lesion was not resected but was treated with radiation. HGAP is generally solitary and this case is of interest due to possible drop met, or just the presence of multiple CNS tumors.

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Concurrent contralateral oligodendroglioma and IDH-mutant astrocytoma

S Schwartz, B White, E Orrego-Gonzalez, H Varma, E Uhlmann, R Vega
Beth Israel Deaconess Medical Center

Background: Multicentric gliomas are rare, and the incorporation of molecular testing into the diagnosis of brain tumors provides us with an opportunity to more completely understand the pathogenesis of these rare tumors. To further understand the origin of these tumors, we present the case of a 39-year-old man presenting with a tonic-clonic seizure beginning in the right hand.

Methods: Imaging on admission demonstrated two different lesions located in the left parietal lobe and in the right insular region. He underwent an initial awake craniotomy (AC) for

the left parietal lesion followed by a second AC to address the right insular lesion.
Results: Neuropathological evaluation of the left parietal biopsy demonstrated an astrocytoma, IDH-mutant, grade 3. Immunohistochemistry demonstrated mutant IDH1 (R132H) staining, loss of ATRX expression, and overexpression of p53. Neuropathological evaluation of the right insular biopsy demonstrated an oligodendroglioma, IDH-mutant and 1p/19q codeleted, grade 2. Immunohistochemistry demonstrated mutant IDH1 (R132H) staining, preservation of ATRX expression, and p53 expression in a subset of cells, consistent with a wild-type pattern. Cytogenetic analysis demonstrated 1p/19q codeletion.
Conclusions: This case is the second such multicentric glioma demonstrating an IDH-mutant astrocytoma and IDH-mutant and 1p/19q codeleted oligodendroglioma in contralateral hemispheres reported in the literature. The findings in these cases, paired with multiple cases of morphologically- and molecularly-defined biphasic oligodendroglioma and astrocytoma, give additional support to the possibility of a shared precursor within IDH-mutated tumors.

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Adult pilocytic astrocytoma with rare ERC2::RAF1 fusion

R Teixeira-Yokoda, M Vij, J Crary, T Richardson, J Walker, M Umphlett, N Tsankova
Icahn School of Medicine at Mount Sinai

Background: Pilocytic astrocytoma (PA) is mostly a pediatric tumor with most patients diagnosed under the age of 20. These tumors occur throughout the neuraxis, most frequently located in the cerebellum and optic chiasm, and are associated with mitogen-activated protein kinase (MAPK) pathway activating fusions or mutations. We report a rare adult cerebellar pilocytic astrocytoma in a 24-year-old female with history of factor V Leiden mutation, which displayed unusual necrotic appearance and carried a previously undescribed ERC2::RAF1 fusion.
Methods: Case report
Results: The patient presented with sinusitis-like headache and left posterior neck pain. Radiology confirmed a 3.6 x 3.4 x 3.3 peripheral rim-enhancing lesion in the left cerebellum, suspicious for an abscess. Intra-operatively, the lesion expressed purulent-like material and frozen section revealed necrosis with neutrophils, although no microorganisms were subsequently cultured from this fluid. Permanent H&E sections disclosed a moderately cellular glial neoplasm with piloid features and low proliferative index, diffusely positive for GFAP and OLIG2. Rosenthal fibers were rare, and a neuronal element was absent. Exuberant areas of ischemic-type necrosis in association with thrombotic vessels were present throughout, possibly related to the patient's Leiden V mutation, complicating clinicopathological differential considerations. Next generation DNA sequencing using hybridization capture technology detected a fusion between RAF1 and ERC2.
Conclusions: RAF1 is a serine/threonine kinase, activating downstream MAPK pathway oncogenic signaling. ERC2 regulates neurotransmitter release but its function in cancer is unknown. Activating mutations or fusions in MAPK pathway members have been discovered in the majority of PAs, with KIAA1549-BRAF fusions found in up to 90% of cerebellar PAs. The rare ERC2::RAF1 fusion, likely representing an activating MAPK pathway oncogenic driver, has been reported in gangliogliomas, but to our knowledge this is the first report in a pilocytic astrocytoma. An association between ERC2::RAF1 fusions and factor V Leiden mutation in pilocytic astrocytomas remains to be established.

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Hemispheric high-grade glioma with H3 K27M mutation: A rare presentation of diffuse not-so-midline glioma

K Zhang[1], B Ramlal[1], K Schreck[1], J Weingart[1], K Aldape[2], C Eberhart[1], C Lucas[1]
[1]Johns Hopkins University; [2]National Institute of Health

Background: H3 K27M mutation is a diagnostic hallmark of diffuse midline gliomas (DMG), which are aggressive brain tumors involving midline structures.
Methods: We describe a H3 K27M-mutant high-grade glioma (HGG) arising in the frontal lobe of a 46-year-old female presenting with seizure.
Results: MR imaging revealed a 4.0 cm enhancing mass. Imaging from 3 years prior demonstrated mild abnormality in the same area. No involvement of midline structures was apparent. Histology revealed a hypercellular glial tumor with microvascular proliferation and necrosis. Tumor cells were immunoreactive for GFAP and OLIG2, negative for IDH1 R132H or BRAF V600E mutant proteins, and had retained ATRX and p53 expression. Synaptophysin immunostaining demonstrated mixed solid and infiltrative growth. There was immunoreactivity for H3 K27M mutant protein along with loss of H3K27me3 expression. Next-generation sequencing identified H3F3A p.K27M (variant allele frequency 42%), NF1 p.E1192* (85%) and TP53 p.E171* (9%) mutations. While the tumor harbored gain of chromosome 7, it lacked chromosome 10 loss, TERT promoter mutation, EGFR amplification, or IDH1/2 mutations. DNA methylation profiling classified the tumor as "DMG, H3 K27-altered" with a high confidence score. However, given the lack of midline structure involvement, the integrated diagnosis was "HGG with H3 K27M mutation, NEC". The patient received hyperbaric oxygen and vitamin therapy in lieu of standard adjuvant therapy. Follow-up imaging at 3- and 6-months after surgery was concerning for tumor progression.
Conclusions: We report a hemispheric H3 K27M-mutant HGG with methylation signature aligning with "DMG, H3 K27-altered". According to the WHO Classification, diagnosing DMG requires histologic evidence of diffuse growth and involvement of midline structures. Cortical H3 K27M-mutant gliomas exhibiting solid growth have apparent better survival, but high-grade diffuse examples have not been reported to our knowledge. This case further emphasizes the need to consider

other entities beyond "glioblastoma, IDH-wildtype" in the differential of IDH-wildtype HGG.

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Intramedullary tumor in adult with 1p/19q codeletion, FGFR1 mutation and DNA methylation profile of diffuse leptomeningeal glioneuronal tumor

P Nisson[1], T Perry[2], K Aldape[3], S Bannykh[2], X Fan[2]
[1]Department of Neurosurgery, Cedars-Sinai Medical Center; [2]Cedars-Sinai Medical Center; [3]2 National Cancer Institute

Background: Proper classification is vital in the prognostication and treatment strategy of tumors. The updated 2021 World Health Organization Classification of CNS Tumors emphasizes molecular mutational profiling in this process, often representing a defining characteristic for categorization. However, a precise genetic and histological profile may contain features that overlap between various tumors, making a definitive diagnosis uncertain as illustrated in this case.
Methods: Clinical Case A 37-year-old female developed bilateral lower extremity sensory-motor changes over 3 years. MRI revealed an expansile poorly defined T1 post-gadolinium enhancing, intramedullary T5-6 spinal cord mass, bright on T2WI and dark on T1WI. No other lesions or leptomeningeal involvement were seen.
Results: The resection specimen revealed an infiltrative growth with a lobular arrangement of oligodendrocytes-like cells which were positive for MAP2, OLIG2, synaptophysin, and negative for IDH1, BRAF V600E, and EMA immunohistochemically. No eosinophilic granular bodies or Rosenthal fibers were noted. FISH showed 1p/19q co-deletion. NGS showed two-point mutations in FGFR1, and mutations in MAP3K1 and SETD2. Chromosomal microarray revealed a combined loss of 1p/19q and gain of 1q with no rearrangements in BRAF. DNA methylation profiling (DNA MP) demonstrated a signature of diffuse leptomeningeal glioneuronal tumor (DLGNT), methylation class 2.
Conclusions: Our findings expose a conundrum in determining which genetic and morphologic findings most strongly correlate with the correct nosology, prognosis, and treatment options. FGFR1 mutations are seen in DNET, DLGNT, rosette-forming glioneuronal tumor (RGNT), and pilocytic astrocytoma. However, the presence of enhancement rules out DNET, and morphology is incompatible with DNET, RGNT and pilocytic astrocytoma. Similarly, extraventricular neurocytoma does not share a MAP3K1 mutation while SETD2 mutation is not entity specific. DNA MP yielded a match with DLGNT though somewhat incongruent radiographic findings were present. These observations suggest that DLGNT may in fact originate from an intramedullary lesion with a propensity for leptomeningeal dissemination.

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Clinically distinct circumscribed H3 K27M-mutant gliomas and glioneuronal tumors: A report of two cases

D BeDell, G Fitzpatrick, R Galvin, D Guillaume, C Ozutemiz, C Moertel, L Chen
University of Minnesota

Background: Circumscribed gliomas with H3 K27M mutations are rare and present a diagnostic challenge, as they can be difficult to distinguish from diffuse midline glioma, H3 K27-altered (DMG). Differentiating between these tumor types is prognostically significant, as circumscribed H3 K27M-mutant gliomas have an intermediate prognosis between DMG and histologically comparable H3-wildtype circumscribed gliomas.
Methods: In this report, we present two clinicopathologically unique cases of circumscribed H3 K27M-mutant tumors, one glioma and one glioneuronal tumor, with distinct histologic appearances and clinical scenarios.
Results: The first case involves a 73-year-old woman with a cerebellar tumor that remained quiescent for 32 years before rapidly growing and requiring resection. This circumscribed glioma exhibited piloid morphology with focal anaplastic features and was confirmed to have the H3 K27M mutation by immunohistochemistry. Whole genome methylation profiling excluded high-grade astrocytoma with piloid features. The second case involves a 4-year-old girl with a glioneuronal tumor in the cervical spinal cord that showed both glial and neuronal components reminiscent of a ganglioglioma. Leptomeningeal dissemination was identified within one year of resection, and the patient exhibited a relapsed/refractory treatment course until stability was achieved on everolimus targeted therapy. Due to more recent subtle concerns for tumor progression, next-generation sequencing was performed, retrospectively identifying the H3 K27M mutation.
Conclusions: These cases further highlight the diverse clinicopathologic features of circumscribed H3 K27M-mutant gliomas and glioneuronal tumors, and demonstrate the importance of accurate diagnosis in this unique subset of tumors, which typically have an intermediate prognosis.

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An Unusual High-Grade Diffuse Glioma with Hybrid Molecular Features of IDH-mutant and IDH-wildtype High-Grade Astrocytoma

B Ebner[1], C Ida[1], K Aldape[2], C Giannini[1], R Jenkins[1], R Vaubel[1]
[1]Mayo Clinic; [2]National Cancer Institute

Background: Astrocytoma, IDH-mutant and glioblastoma, IDH-wildtype demonstrate differing clinical characteristics and outcomes. These entities arise from distinct driver mutations which result in unique molecular and epigenic alterations.

Methods: We describe a case from a 75-year-old male who underwent resection of a rim-enhancing T2 hyperintense mass involving the left temporal lobe.
Results: The histologic sections demonstrated a mitotically active infiltrating glioma, composed of tumor cells demonstrating predominantly fibrillary morphology. Microvascular proliferation and large areas of necrosis were present. Immunohistochemical staining showed the tumor cells to be diffusely positive for IDH1-R132H in multiple tissue blocks. Expression of ATRX was retained and p53 overexpressed. Targeted next generation sequencing confirmed the presence IDH1 p.R132H mutation (c.395G>A, variant allele frequency: 26.4%) and identified a TERT promoter C250T mutation (-146C>T, variant allele frequency: 41%). The consistent IDH1 immunostaining across the tumor and the IDH1 variant allele frequency supported IDH1 mutation as a clonal driver event. By chromosomal microarray, the tumor was tested twice on re-extracted DNA and showed alterations meeting the diagnostic criteria of molecular glioblastoma, IDH-wildtype, including gain of chromosome 7, loss of chromosome 10, and EGFR amplification. Additional alterations more commonly associated with glioblastoma, IDH-wildtype were also observed including amplification of CDK4 and MDM4, and homozygous deletion of PTEN. By methylation profiling, the tumor was not definitively classified, with a low-confidence match to glioblastoma, IDH wildtype, subclass RTK II (methylation class family Glioblastoma, IDH wildtype, calibrated score 0.68) but embedded with high-grade astrocytoma, IDH-mutant samples on Uniform Manifold Approximation and Projection. Copy-number plot was in keeping with the chromosomal microarray findings.
Conclusions: While EGFR amplification has been rarely reported in astrocytoma, IDH-mutant, this tumor fulfills all key histologic and molecular criteria of glioblastoma, IDH-wildtype while harboring an IDH1 mutation. The tumor was considered to represent a hybrid diffuse glioma, with IDH1 mutation and molecular features of glioblastoma, IDH-wildtype.

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Genomic Anatomy/Architecture of CDKN2A/B and MTAP Deletions in Brain Tumors

B Ebner, C Ida, A Raghunathan
Mayo Clinic

Background: CDKN2A/CDKN2B homozygous deletion is a molecular prognostic biomarker of multiple brain tumor types and is unreliably evaluated using p16 immunostain as a surrogate marker. MTAP immunostain has emerged as a surrogate marker of CDKN2A homozygous deletion because MTAP gene is approximately 100kb telomeric of CDK2NA and appears frequently deleted in tumors harboring CDKN2A homozygous deletion. The genomic anatomy/architecture of MTAP and CDKN2A/B deletions remains unclear.
Methods: Herein, we assess the frequency and correlation between CDKN2A/B and MTAP deletions from 349 brain tumors by Oncoscan chromosomal microarray. Additionally, p16 and MTAP immunostains were performed on a subset of cases to assess the correlation between protein expression and CDKN2A and MTAP copy-number status.
Results: Cases included glioblastoma, IDH-wildtype (n = 203), astrocytoma, IDH-mutant (n = 82) oligodendroglioma, 1p/19q-codeleted and IDH-mutant (n = 49), diffuse midline glioma, H3 K27M-altered (n = 6), pleomorphic xanthoastrocytoma (n = 7) and pilocytic astrocytoma (n = 3). Of the 217 tumors with CDKN2A/B deletions, 216 (99.5%) had concurrent MTAP deletions. All tumors with CDKN2A/B heterozygous deletion (n = 60) had concurrent MTAP heterozygous deletion. Tumors with CDKN2A/B homozygous deletion (n = 157; 150 with deletion of both genes) predominantly demonstrated MTAP homozygous deletion (n = 116; 73.9%), which is expected to translate in loss of MTAP immunostain expression; a subset of CDKN2A/B homozygously deleted tumors harbored MTAP heterozygous deletion (n = 40; 25.4%), which would be predicted to result in preserved MTAP immunostain expression and failure to indicate presence of CDKN2A/B homozygous deletion. Isolated MTAP deletions without concurrent CDKN2A/B deletions were not observed. By immunohistochemistry, 9 of 12 IDH-mutant tumors with CDKN2A homozygous deletion showed MTAP loss and 32 of 34 tumors without CDKN2A homozygous deletion retained expression of MTAP.
Conclusions: CDKN2A/B and MTAP homozygous deletion frequently but not always co-occur and is in keeping with our included immunohistochemical findings and the reported 70-90% sensitivity for MTAP immunostain as a surrogate marker for CDKN2A homozygous deletion.

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Expanding the spectrum of TERT promoter non-canonical sequence variants in brain tumors: A case series

D Cook[1], N Saha[1], T Koganti[1], Z Abdullaev[2], K Aldape[2], K Fung[3], S Powell[4], D Trivedi[5], R Vaubel[1], C Giannini[1], R Jenkins[1], C Ida[1]
[1]Mayo Clinic; [2]National Cancer Institute; [3]University of Oklahoma Health Sciences Center; [4]Houston Methodist Hospital; [5]Ochsner Medical Center

Background: The C228T and C250T are canonical TERT promoter (TERTp) activating hotspot missense mutations. Rare non-canonical TERTp activating duplications have been recently reported.
Methods: We describe five cases with non-canonical TERTp variants detected using targeted neuro-oncology NGS panels (2018-2022) from a clinical reference laboratory: c.-100_-79dup (n = 2), c.-92_-91insGGCGGCCCCGCCCCTTCCTTTC, c.-118_-117insTCCCCGGCCCAGCCCCTTCCGGG and c.-123_-76dup. Three (of 5) cases also had chromosomal microarray, and one case had methylation array profiling data.
Results: All non-canonical TERTp variants occurred at a variant allele frequency suggestive of somatic clonal origin (median, 25%; range, 18-30), and in hemispheric IDH/H3-wildtype

high-grade diffuse astrocytic gliomas of adults (median age 68 years; range 61-88). The c.-100_-79dup has been recurrently reported in glioblastoma, IDH-wildtype (GBM-IDHWT) and functionally shown to be activating. This variant occurred in the two tumor resection specimens without grade 4 features: one had NF1/TP53/PIK3R1 mutations, and chromosome 9/22 gain/14 loss/17 copy-neutral loss of heterozygosity; the other had EGFR/PTEN/STAG2 mutations, and methylation profile matching to GBM (1.0)/GBM, RTK1 (0.76) on v11b6 and to pedHGG_RTK2 on v12b6 (0.91)/NCI (0.98) classifiers, with chromosome 7 gain/10 loss on copy-number plot. On UMAP, pedHGG_RTK2 is a cluster in close vicinity to GBM, RTK1 and reportedly enriched for TERTp mutations. The remaining TERTp non-canonical variants occurred in GBM-IDHWT with grade 4 features and have not been reported. The size and sequence of such variants suggest they may be functionally equivalent to C228T mutation and generate favorable TERTp activation motif conformations like previously reported activating duplications. The tumor with c.-92_-91ins had NF1/PIK3CA mutations; the case with c.-123_-76dup had PTEN/MYCN mutations and chromosome 7 gain/10 loss/CDK4, MDM2 amplification; and the tumor with c.-118_-117ins had a CBL mutation.

Conclusions: Our findings expand the spectrum of TERTp non-canonical variants in brain tumors and further suggest that they typically occur in tumors with features of GBM-IDHWT as a clinically/diagnostically relevant alternative TERTp activating mechanism.

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IDH-Mutant Astrocytomas Demonstrate Globally Decreased CpG Island Methylation After Temozolomide: A Matched Cohort Study

G Chamberlin[1], D Trembath[2], K Jones[1], Y Rauf[2], S Khagi[3]
[1]Duke University Medical Center; [2]University of North Carolina Hospitals; [3]Dartmouth Cancer Center

Background: Methylation profiling can provide unique insights into the development and progression of CNS neoplasms. Multiple large studies have documented numerous genetic and epigenetic alterations in the progression of adult-type diffuse gliomas. However, these studies do not use matched pre- and post-treatment specimens from the same cohort of patients. This study aims to improve our understanding of the natural history of adult-type diffuse gliomas by examining the methylome of matched CNS neoplasm specimens.

Methods: A cohort of 14 patients was retrospectively identified at a large academic cancer center, each of whom underwent at least 2 surgical procedures for a primary CNS neoplasm. Diagnoses included glioblastoma, astrocytoma, oligodendroglioma, and central neurocytoma. Among these patients, 33 tumor samples were collected, including an initial diagnostic specimen and at least one recurrence per patient. DNA was extracted from each tissue specimen and subjected to methylation analysis. Each of the four tumor types was analyzed separately. Methylation signatures were compared between diagnostic samples and recurrences using linear modeling in Limma (v. 3.50.3).

Results: Among IDH-mutant astrocytomas, 915 of 10,000 probes demonstrated a significant decrease in methylation beta value following treatment (BH-adjusted p-value < 0.05), while only 6 probes showed significantly increased methylation. Probes showing altered methylation were widely distributed across the genome. Unsupervised hierarchical clustering demonstrated that pre-treatment specimens were more similar to pre-treatment specimens from other patients than they were to post-treatment specimens from the same patient. Clustering appears largely driven by temozolomide exposure. There was no significant change in the methylation profile in the glioblastoma, oligodendroglioma, or central neurocytoma groups.

Conclusions: Methylation profiling of pre- and post-treatment glioma specimens may identify specific avenues for tumor progression despite (or due to) treatment with temozolomide and radiation. Further study of the differentially methylated probes in IDH-mutant astrocytomas may allow for the development of targeted therapeutics in recurrent disease.

PLATFORM 5: Neurodegenerative: FTLD, Lewy Body, Parkinson, Other

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A Drosophila model of chemotherapy-related cognitive impairment

M Torre[1], M Feany[2]
[1]Brigham and Women's Hospital and Harvard Medical School; [2]Brigham and Women's Hospital and Harvard Medical School

Background: Chemotherapy-related cognitive impairment (CRCI) is a common adverse effect of chemotherapy that results in significant patient morbidity. However, the pathophysiology of CRCI is incompletely understood. As a fundamental step towards dissecting the mechanistic pathways underlying CRCI, we have validated a Drosophila model that demonstrates cognitive and neuropathologic features described in chemotherapy patients.

Methods: Five-day-old adult Drosophila were administered a diet containing cisplatin (10, 100, or 500 ug/ml) or water control for three days and aged to 10, 20, or 30 days. General neurologic function and memory/learning were then assessed using the climbing assay and taste memory assay, respectively. Brains were formalin-fixed and paraffin-embedded and evaluated for histologic and immunohistochemical evidence of neurodegeneration, oxidative stress, and DNA damage. Comparisons between groups were assessed using one or two-way ANOVA with Tukey's multiple comparisons test ($p < 0.05$ considered statistically significant).

Results: Cisplatin-treated Drosophila had impaired performance on the climbing assay (a general neurologic readout) and taste memory assay. We also observed that neurodegeneration, oxidative stress, and DNA damage in the brain were typically age and cisplatin dose dependent. Aged Drosophila that had been given high-dose cisplatin showed increased vacu-

olar neurodegeneration and apoptotic cells in the brain (assessed by caspase activation). Oxidative damage (determined by expression of GstD1 and Puc) was elevated in aged Drosophila with history of intermediate and high-dose cisplatin. Increased DNA damage (indicated by pH2AV) was seen across ages in Drosophila treated with intermediate and high-dose cisplatin.

Conclusions: We validate a working Drosophila model of CRCI that recapitulates features of the cognitive, radiologic, and molecular changes observed in human chemotherapy patients. Drosophila administered cisplatin show impaired neurologic function and memory/learning and increased neurodegeneration, oxidative stress, and DNA damage in the brain. This Drosophila model can be used for high throughput genetic/pharmacologic screening to identify therapeutically targetable pathways contributing to CRCI.

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Thiamine and biotin supplementation rescues oligodendrocyte pathology in Huntington Disease

O Al Dalahmah[1], R Lim[2], J Wu[2], J Goldman[1], L Thompson[2]
[1]Columbia University; [2]University of California Irvine

Background: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease that leads to neuronal loss and gliosis in multiple brain regions. White matter pathology is one of the earliest radiologically detectable signs in HD, manifesting years before clinical signs and symptoms appear. Studies from model organisms incriminated oligodendrocyte (OL) maturation defects in HD pathology. However, systematic analyses of OL pathology in human and murine HD have been lacking.

Methods: Here we used single nucleus RNA sequencing (snRNAseq) to define the molecular pathology of OL-lineage cells in human HD post-mortem tissue and R6/2 HD mice. We analyzed 66 human samples from the caudate, accumbens, and cingulate cortex (n = 29 donors: 3 grade-I, 4 grade-II, 4 grade-III, 3 grade-IV, 5 juvenile-onset HD, and 10 matched controls). We measured CAG repeat lengths to correlate with transcriptional findings. We also analyzed the cortex and caudate-putamen from 8-week and 12-week-old R6/2 HD and control mice (n = 3/group), and conducted complementary lipidomic and chromatin-accessibility studies. In mechanistic validation studies, we treated control and R6/1 HD mice with thiamine-biotin for 7 weeks before analyzing their striata using snRNAseq.

Results: We identified transcriptional signatures supporting that OLs and OL precursors (OPCs) were arrested in intermediate maturation states. In fact, the expression of OL-lineage regulators OLIG1 and OLIG2 in human OPCs was negatively correlated with CAG repeat length. Oligodendrocyte maturation defects spanned the human HD cingulate cortex, caudate, and the accumbens, and were recapitulated in murine cortical and striatal OLs. Our data implicated glucose and lipid metabolism in abnormal cell maturation and identified PRKCE and Thiamine Pyrophosphokinase 1 (TPK1) as central genes that were decreased in HD brains. Thiamine/biotin treatment of R6/1 HD mice to compensate for TPK1 dysregulation restored OL maturation.

Conclusions: Our insights into HD OL pathology spans multiple brain regions across two species, and link OL maturation deficits to abnormal thiamine metabolism.

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Aging-related tau astrogliopathy (ARTAG) and cognitive outcomes in community-based older persons

S Agrawal[1], L Yu[2], S Leurgans[2], A Kapasi[2], L Barnes[2], D Bennett[2], P Boyle[2], J Schneider[2]
[1]Rush Alzheimer's Disease Center, Rush University Medical Center; [2]Rush University Medical Center

Background: Gray matter aging-related tau astrogliopathy (GM ARTAG) is 4R tau astrocytic pathology, recognized commonly in individuals >60 years old. However, the association of GM ARTAG with Alzheimer's dementia and decline in multiple cognitive systems is unclear.

Methods: Participants (n = 427, mean age-at-death=89 years, women=66%) were enrolled from 3 ongoing community-based cohorts with annual cognitive testing. At autopsy, overall and regional GM ARTAG severity from the superior frontal, temporal tip, and amygdala regions were graded from 0 (none) to 6 (severe) using immunohistochemistry with antibody specific to PHF-tau. AD and other age-related pathologies were evaluated. The association of GM ARTAG with Alzheimer's dementia was examined using logistic regression models, and its association with cognitive decline was examined using linear mixed models controlling for age, sex, education, a pathologic diagnosis of AD, and other common age-related pathologies.

Results: Overall GM ARTAG was present in 72% of the participants. The proportion with GM ARTAG pathology varied across the three regions, with amygdala being the predominant region (68%), followed by the anterior temporal tip (55%) and the superior frontal region (31%). GM ARTAG in each of the three regions was moderately related to a pathologic diagnosis of AD (ρs range from 0.39-0.53). GM ARTAG in the superior frontal region, but not the amygdala or temporal tip, was associated with higher odds of Alzheimer's dementia (OR = 1.66, 95%CI=1.11–2.49). GM ARTAG in the superior frontal was also associated with a higher rate of decline in global cognition (Est=-0.014, SE = 0.006, p = 0.029), and specifically episodic memory and semantic memory. GM ARTAG in the temporal tip and amygdala were not associated with cognitive decline.

Conclusions: GM ARTAG pathology in the superior frontal cortex is common in community-dwelling older persons and is associated with Alzheimer's dementia and decline in global

cognition, even after controlling for a pathologic diagnosis of AD and other pathologies.

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Clinicopathologic features of a novel star-like transactive response DNA-binding protein 43 (TDP-43) pathology

A Carlos, S Koga, R Gatto, N Pham, R Petersen, C Jack, V Lowe, J Whitwell, D Dickson, R Reichard, J Parisi, K Josephs
Mayo Clinic

Background: Transactive response DNA-binding protein 43 (TDP-43) pathology in frontotemporal lobar degeneration (FTLD) and Alzheimer's disease (AD) is categorized based on morphologic characteristics of inclusions. Types A-E have been described in FTLD,while type-α (reminiscent of FTLD-TDP Type A) and type-β (colocalizing with tau neurofibrillary tangles) have been described in AD. Here we describe clinicopathologic features of a novel TDP-43 inclusion.
Methods: Amygdala slides of 131 cases with varying ages at death and AD neuropathologic changes were immunostained and screened for TDP-43 pathology. Seventy-five(57%) TDP-43-positives were found and subsequently typed based on predominant morphology of the inclusions. Seven of the 75 cases(9%) showed atypical TDP-43 inclusions not previously described and could therefore not be typed.
Results: All seven (3 males,4 females) showed star-like or crown-like TDP-43-immunoreactive inclusions, with occasional coiled body-like TDP-43 inclusions, predominantly in superficial (subpial) amygdala and less in subependymal and/or white matter regions. All had died at an extremely old age (median:99.8 years[IQR:93.5,101.4]) from heart failure, pneumonia, cancer or severe fall-induced injuries, after a prolonged agonal period. One carried the apolipoprotein E ε4 allele. None had dementia prior to death, but instead normal cognition or mild cognitive impairment of the amnestic type. MRI scans from five cases showed moderate generalized atrophy, marked in temporal lobe, and leukoaraiosis. Three had fluorodeoxyglucose-PET, with one showing focal progressive left medial temporal hypometabolism. Pathologically, the star-like TDP-43 inclusions were not detected in neither hippocampal nor frontal regions. Aging-related tau astrogliopathy (ARTAG) was frequently found. Other copathologies included low-ADNC (n = 1), intermediate-ADNC (n = 3) and primary age-related tauopathy(n = 3). Lewy body disease (brainstem-predominant[n = 3], transitional[n = 1]) and variable cerebrovascular pathology, including cerebral amyloid angiopathy and arteriolosclerosis with occasional large infarcts or microinfarcts, were also present.
Conclusions: We identified a novel TDP-43 pathology with star-like morphology associated with very old age (superaging) with a relatively homogeneous clinicopathologic picture, possibly representing a novel, true aging-related TDP-43 pathology.

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Multi-modal Proteomic Characterization of Lysosomal Function and Proteostasis in Progranulin-Deficient Neurons

S Hasan[1], M Fernandopulle[2], S Humble[2], A Frankenfield[3], H Li[3], R Prestil[4], K Johnson[2], B Ryan[5], R Wade-martins[5], M Ward[2], L Hao[3]
[1]NIH+UCL+AU/UGA; [2]NIH; [3]George Washington University; [4]University of Cambridge; [5]University of Oxford

Background: Mutations in the GRN gene, which encodes the intralysosomal protein progranulin (PGRN), reduce its expression and cause frontotemporal dementia (FTD). PGRN loss results in abnormalities in autophagy, lysosomal proteolysis, and lipid metabolism. However, both the molecular function of PGRN within lysosomes and the impact of its loss on lysosomal biology remains unclear.
Methods: Here, we leveraged multi-faceted proteomic techniques to comprehensively characterize how PGRN deficiency changes the molecular and functional landscape of neuronal lysosomes. We characterized lysosome composition and its interactome in both human induced pluripotent stem cell (iPSC)-derived human glutamatergic neurons (i3Neurons) and mouse brain tissues. Using dynamic stable isotope labeling using amino acids in cell culture (dSILAC) proteomics, we measured global protein half-lives in human neurons for the first time, characterizing the impact of progranulin loss on neuronal proteostasis.
Results: Collectively, these studies indicate that PGRN loss impairs the lysosome's degradative capacity, in part through alkalinization of lysosomal pH. These abnormalities occur despite upregulation of lysosomal v-ATPases and lysosomal hydrolases in PGRN deficient human neurons and PGRN deficient mouse brains. Consistent with impairments in lysosomal function, GRN deficient neurons had pronounced alterations in protein turnover, including slower turnover of cathepsins, proteins related to supramolecular polymerization, and a host of proteins implicated in familial neurodegenerative diseases.
Conclusions: Together, these results implicate PGRN as a critical regulator of lysosomal pH and degradative capacity, which in turn influences global proteostasis in neurons. Our findings further indicate a convergence of lysosomal dysfunction pathways in neurodegenerative diseases.

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Reactivity drives astrocytic tau accumulation in FTLD-tau

K Fiock[1], J Hook[1], M Shin[2], M Hefti[1]
[1]University of Iowa Department of Pathology; [2]University of Iowa Pathology Department

Background: Toxicity caused by abnormal accumulations of tau protein is the final step in the pathway of many neurode-

generative diseases, including Alzheimer's disease (AD), frontotemporal lobar degeneration (FTLD-tau), chronic traumatic encephalopathy (CTE), and other tauopathies. Though this process has been extensively studied in neurons in the context of AD, the mechanisms that drive this same process in astrocytes, and how that in turn drives diseases like FTLD-tau, remain poorly understood.

Methods: Using a combination of human tissue and human stem cell-derived (hSC) astrocytes, we sought to uncover the origin of astrocytic tau in FTLD-tau and the potential candidate mediators of tau accumulation in astrocytes in vitro. We used RNA in situ hybridization and immunofluorescence on cases of AD, PSP, and CBD to compare total amounts of tau mRNA between diseases and between astrocytes with and without tau pathology in PSP. We then used hSC-derived astrocytes to assess their ability to take up and degrade different isoforms of tau.

Results: We saw no significant differences in astrocytic tau expression between diseases with and without astrocytic tau pathology or between individual astrocytes with and without pathology in our PSP cohort. We also found that both control and reactive astrocytes in vitro preferentially take up 4R tau, but only reactive astrocytes exhibit significantly impaired degradation. Across diseases, astrocytes with tau pathology do not show elevated markers associated with the neurotoxic A1 phenotype.

Conclusions: Taken together, this data suggests that neuronal and astrocytic tau in FTLD-tau shares a common neuronal origin, and that the preferential uptake and subsequent impaired degradation of 4R tau may explain why 4R accumulation is more common in astrocytes. These results also suggest that tau uptake by astrocytes may have a protective function. Future work focused on characterization of the neuroprotective A2 phenotype is necessary to further elucidate the role of astrocytes in FTLD-tau.

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Custom made TDP-43 antibodies are biomarkers to differentiate subtypes of LATE-NC with or without ADNC

Q Mao[1], L Robinson[1], E Goold[1], J Kofler[2], P Nelson[3]
[1]University of Utah; [2]University of Pittsburgh; [3]University of Kentucky

Background: TAR DNA binding protein of 43 kDa (TDP-43) was initially associated with frontotemporal lobar degeneration (FTLD). TDP-43 is also prevalent in aging brains and is associated with cognitive impairment, and this disease has been designated "limbic predominant age-related TDP-43 encephalopathy" (LATE). LATE neuropathologic change (LATE-NC) is often comorbid with Alzheimer's disease (AD) neuropathologic change (ADNC). Using novel, custom made monoclonal antibodies (MAbs) against TDP-43, we previously found that the TDP-43 species in ADNC brains differ in their molecular composition from those in FTLD brains with TDP-43 inclusions (FTLD-TDP).

Methods: In this study, we further analyzed the distribution of the new TDP-43 species in ADNC brains using a novel anti-TDP-43 antibody, MAb#14. Control antibodies included the commercial p409/410 MAb, and MAb#9, the species-specific antibody for FTLD-TDP that we have characterized.

Results: In high ADNC brains with LATE-NC, the limbic areas showed significantly more MAb#14 positivity, including dystrophic neurites, neuronal cytoplasmic inclusions, and neurofibrillary tangle-like and plaque-like inclusions, than p409/410 positivity. In high ADNC brains without LATE-NC, the limbic areas also showed abundant MAb#14 positivity. The cortex, deep grey matter, and hippocampus showed abundant plaque-like MAb#14-positivity in high ADNC brains both with and without LATE-NC. MAb#14 revealed more co-localization with tangles than p409/410 MAb. MAb#14 was partially colocalized with beta-amyloid in plaques, which has not been reported before. MAb#9 revealed slightly more TDP-43 inclusions than the p409/410 MAb in ADNC with LATE-NC brains; however, both MAb#9 and p409/410 MAb were negative in ADNC without LATE-NC. Interestingly, in LATE-NC brains without ADNC, p409/410 MAb-, MAb#14-, and MAb#9-positivity were colocalized, indicating that the ADNC-related TDP-43 species were absent. Lastly, MSD immunoassays with MAb#14 detected higher plasma levels of TDP-43 in AD than in FTLD patients.

Conclusions: Our findings demonstrate that the MAb#14-positive TDP-43 species is ADNC-specific and can be a biomarker of TDP-43 proteinopathy in ADNC.

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Impaired Proteostasis in Multisystem Proteinopathy due to Pathogenic VCP Variants

E Lee[1], J Phan[1], B Creekmore[1], A Nguyen[2], D Bershadskaya[1], N Darwich[1]
[1]University of Pennsylvania; [2]Mayo Clinic

Background: Pathogenic variants in VCP cause multisystem proteinopathy (MSP), a disease characterized by multiple clinical phenotypes including inclusion body myopathy, Paget's disease of the bone, and frontotemporal dementia (FTD). How such diverse phenotypes are driven by pathogenic VCP variants is not known.

Methods: Genetically engineered cell lines were studied using molecular and pharmacologic approaches to determine how pathogenic VCP variants affect turnover of TDP-43 protein aggregates. CRISPR-Cas9 was used to generate knock-in cell lines harboring MSP variants to study how mutant VCP alters cellular phenotypes. We also developed a cellular model of TDP-43 proteinopathy where proteostatic stress results in the formation of insoluble TDP-43 aggregates.

Results: Cells harboring MSP variants or cells treated with VCP inhibitor exhibited decreased clearance of insoluble TDP-43 aggregates. Moreover, we identified four novel compounds that activate VCP primarily by increasing D2 ATPase activity,

and that pharmacologic VCP activation appears to enhance clearance of insoluble TDP-43 aggregates.
Conclusions: Our findings suggest that VCP function is important for protein homeostasis, that MSP may be the result of impaired proteostasis, and that VCP activation may be potential therapeutic by virtue of enhancing the clearance of protein aggregates.

PLATFORM 6: Tumors: Nonglial

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Inducing neural maturation in medulloblastoma by targeting EZH2

M Snuderl[1], M Snuderl[1], K Galbraith[1], D Tylawsky[2], B Liechty[3], J Vaynshteyn[4], J Gerwin[4], C Orillac[1], D Boué[5], D Heller[2], G Raju[4]
[1]NYU Langone Health; [2]Memorial Sloan Kettering Cancer Center; [3]Weill Cornell Medicine; [4]Icahn School of Medicine at Mount Sinai; [5]Nationwide Children's Hospital and The Ohio State University

Background: Medulloblastoma (MB) is the most common malignant pediatric brain tumor. Histologically, some MB, particularly the SHH subtype, show nodular architecture composed of sheets of primitive cells and scattered islands of mature cells with more abundant neuropil and low proliferation rate. We hypothesized that spontaneous maturation of MB cells is governed by epigenetic mechanisms and maturation in MB can be induced for therapeutic purposes.
Methods: We performed laser capture microdissection followed by whole transcriptome analysis, spatial transcriptomics using Digital Spatial Profiling, whole genome DNA methylation profiling and ChIP-Seq analysis of mature and primitive areas from 8 human medulloblastoma samples. We developed a genetically-engineered mouse model (GEMM) of SHH medulloblastoma showing spontaneous maturation, in conjunction with either conditional EZH2 genetic ablation or EZH2 (Y641F) overactivation. Finally, we developed a fucoidan-based nanoparticle drug delivery across the blood brain barrier (BBB) for targeted molecular inhibition.
Results: Using whole transcriptome and DNA methylation analysis, we identified ~120 differentially expressed genes between primitive and mature regions with enrichment for genes regulated by H3K4me3 and H3K27me3 or SUZ12 in human SHH nodular/desmoplastic medulloblastoma. ChIP-Seq analysis showed striking differences in H3K27me3 enrichment between primitive and mature medulloblastoma cells including at the EZH2 locus. Medulloblastoma specific EZH2 genetic ablation resulted in diffuse tumor cell differentiation and prolonged survival in mice ($n = 10$ per group, log-rank $p = 0.01$). Conversely, conditional EZH2 (Y641F) activation prevented medulloblastoma differentiation. A fucoidan-based nanoparticle successfully delivered the EZH2 inhibitor (EPZ-6438) across the murine intact BBB to achieve significant extension of mouse survival (median 70 days compared to 21 days in control mice; *$p=0.01$, Mantel-Cox).
Conclusions: Spontaneous maturation in MB is regulated by PRC2 complex and can be induced by inhibition of EZH2. Fucoidan-based nanoparticles allow tumor specific targeted EZH2 inhibitor drug delivery across the BBB extending survival and limiting toxicity.

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Large cell/anaplastic medulloblastoma represents more than one disease entity

T Pietsch, K Krause, E Doerner, T Goschzik
University of Bonn, Department of Neuropathology

Background: Medulloblastoma (MB) with predominant large cell or anaplastic cytological features (LC/A) have been identified to be associated to aggressive clinical course and poor survival. Because cases can show mixed cytologies, these histologically defined MB types were merged in the recent WHO classification of tumors of the CNS to large cell/anaplastic (LC/A) MB.
Methods: In this study, we collected FFPE samples of 130 LC/A MB of all age groups (1-39 years) diagnosed at the DGNN Brain Tumor Reference Center in Bonn, Germany, and performed a comprehensive histological, immunophenotypical, genetic and epigenetic analysis by next-generation panel sequencing (NGS), 450k/850k methylation bead-array hybridization and molecular inversion probe (MIP) technology.
Results: 72% of cases showed predominant anaplastic histology, 28% predominant large cell histology. Methylation-based subtyping and sequencing showed that the cohort of LC/A MB represented different biological entities: 5% WNT-MB, 7% SHH MB-TP53-wildtype, 12% SHH-MB-TP53-mutant, and 76% non-WNT/non-SHH MB (with predominant epigenetic subtype II). High-resolution copy number profiling by MIP demonstrated MYC amplification in 42% of non-WNT/non-SHH MB. MYC amplification was highly associated to infant disease and predominant large cell histology. On the contrary, predominantly anaplastic histology was significantly associated to non-infant disease and MYCN amplification. By NGS we detected recurrent mutations in 37 MB-related genes; the frequency of mutations of individual MB-related genes differed significantly between the histologically and genetically defined MB types.
Conclusions: Our data clearly demonstrate that large cell MB and anaplastic MB represent different biological entities. Therefore, and also in respect to the future development of

targeted treatment approaches to these highly malignant MB types, they should be precisely diagnosed as separate MB entities.

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DNA-methylation subgroups carry no prognostic significance in ATRT-SHH patients treated on clinical trials

Q Tran[1], S Upadhyaya[2], C Billups[3], A Onar-Thomas[3], S Carey[3], M Alom[3], G Robinson[3], D Ellison[3], A Gajjar[3], B Orr[3]
[1]St. Jude Children's Research Hospital; [2]C.S., Mott Children's Hospital, University of Michigan; [3]St. Jude Children's Research Hospital

Background: Three molecular subtypes have been established for atypical teratoid/rhabdoid tumors (ATRT-SHH, ATRT-MYC, and ATRT-TYR). Recent analysis of a registry cohort reported three subgroups of ATRT-SHH (ATRT-SHH-1A, 1B, and 2). ATRT-SHH-1B tumors presented in slightly older patients and demonstrated improved outcome. The clinical and outcome differences in ATRT-SHH molecular subgroups have not been validated in clinical trial cohorts.
Methods: We evaluated a cohort of ATRT-SHH patients treated on St. Jude Children's Research Hospital clinical trials ($n = 39$, SJYC07, SJMB03, SJATRT), a consortium trial ($n = 2$, PBTC-001), and equivalently treated patients on non-protocol treatment plans (NPTP, $n = 2$). Using unsupervised and semisupervised methods applied to DNA methylation data we assigned consensus subgroup labels to 41 cases (14 ATRT-SHH-1A, 11 ATRT-SHH-1B, and 16 ATRT-SHH-2). Clinical and outcome data was evaluated with respect to molecular subgroup.
Results: Age at diagnosis ($p = 0.008$) and tumor site ($p = 0.005$) were statistically different among ATRT-SHH subgroups. We observed enrichment for ATRT-SHH-2 in the infratentorial compartment, and ATRT-SHH-1A and 1B tumors in the supratentorial compartment. No differences were identified with respect to gender, germline cancer predisposition, overall survival (OS), progression free survival (PFS), or event free survival (EFS) among subgroups. Since metastatic status at the time of presentation is known to impact outcome, we investigated OS, PFS, and EFS by SHH subgroup in M0, M+ and MX frontline patients separately and found no significant differences in outcome.
Conclusions: Our findings confirmed the reported age and location differences between the ATRT-SHH subgroups, but we found no prognostic difference between subgroups in patients treated on clinical trials. Our findings suggest that ATRT-SHH subgroup designation should not be used as the basis for trial stratification or clinical decision making at this time. Further research is needed to determine the underlying determinants for the ATRT-SHH molecular subgroups and scenarios in which they may be clinically important.

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Embryonal tumor with multilayered rosettes (ETMR) lacking C19MC and DICER1 alterations versus new CNS embryonal tumor type

E Guney[1], K Mirchia[2], R Lu[2], A Putnam[3], M Alashari[3], H Zhou[3], N Whipple[4], P Chan[4], S Cheshier[3], S Wu[5], D Samuel[5], S Ferris[6], E Pinarbasi[6], M Terry[2], M Pekmezci[2], A Bollen[2], T Tihan[2], K Aldape[7], A Perry[2]
[1]University of California, San Francisco (UCSF); [2]UCSF; [3]Primary Children's Medical Center, University of Utah; [4]Primary Children's Hospital and University of Utah/Huntsman Cancer Institute; [5]Valley Children's Hospital; [6]University of Michigan; [7]NIH/NCI

Background: Embryonal tumor with multilayered rosettes (ETMR) is a distinct CNS neoplasm with primitive cytology, characteristic rosettes, variable neuronal differentiation, strong and diffuse LIN28A immunoreactivity, and either C19MC or DICER1 alterations.
Methods: In this study, clinicopathologic review, targeted next-generation DNA sequencing (NGS), and DNA methylation profiling (DNAMP) were performed on tumors from 6 patients (2 male, 4 female) with a median age of 8 months (range: 9 days to 15 months), that had neither C19MC nor DICER1 alteration, but clustered with ETMR by DNAMP nonetheless.
Results: The tumor was centered in the posterior fossa in 5, with involvement of midbrain, thalamus, and pineal gland in one. While all cases demonstrated features of an embryonal neoplasm, multilayered rosettes were absent in 5 (83%). LIN28A was immunonegative in 2 (33%), focally positive in 2 (33%), extensively positive in 1 (17%), and not performed in 1. While a missense TP53 mutation was identified in a single case, NGS showed no pathogenic alterations in the remaining cases. Two patients are alive at 8- and 12-months post-surgery, and 3 patients died 5-, 7- and 20-months after surgery. One tumor showed complete glioneuronal maturation (i.e. no residual primitive cells) on 3 subsequent specimens following chemotherapy.
Conclusions: In summary, we report 6 CNS tumors of infancy with unusual histopathologic and molecular features. Currently, it is unclear if these cases represent a completely new tumor type/entity or a subtype of ETMR with atypical histopathologic features. Further studies are needed to better characterize this novel CNS embryonal neoplasm.

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Systematic Characterization of Antibody-Drug Conjugate Targets in Central Nervous System Tumors

S Coy[1], J Lee[2], S Chan[3], K Ligon[4], S Santagata[5]
[1]Brigham and Women's Hospital, Harvard Medical School; [2]Harvard Medical School; [3]Brigham and Women's Hospital, Dana-Farber Cancer Institute; [4]Brigham and Women's

Hospital, Dana-Farber Cancer Institute, Harvard Medical School; [5]Brigham and Women's Hospital, Harvard Medical School

Background: Antibody-drug conjugates (ADC) are monoclonal antibodies linked to chemotherapeutic payloads, which enhance the specificity and therapeutic window of cytotoxic drugs by directing them to tumor cells expressing target antigens. Multiple ADC are FDA-approved for solid and hematologic malignancies, including those targeting HER2, TROP2, and Nectin4, with dozens more in development for additional indications. Recently, HER2 ADC (Trastuzumab-Deruxtecan) was found to increase survival and reduce brain metastasis growth in treatment-refractory metastatic breast cancer, even in tumors with low or heterogeneous HER2 expression. This suggests that even low-level ADC target expression by CNS tumors may indicate opportunities for additional trials; however, ADC target expression patterns are unknown for many CNS tumors.
Methods: We analyzed publicly-available RNA-sequencing and proteomic data from the Children's Brain Tumor Network (CBTN/CPTAC) (N = 188 tumors), and GEO RNA-expression datasets (N = 356 tumors) for expression of ADC target antigens in diverse tumor subtypes. We performed semi-quantitative IHC scoring (0-3) for HER2, HER3, Nectin4, TROP2, CLDN6, CLDN18.2, and CD276/B7-H3 on a large panel of human CNS tumors, including glioblastomas (N = 144), oligodendrogliomas (N = 14), anaplastic oligodendrogliomas (N = 16), meningiomas (N = 248), ependymomas (N = 44), pilocytic astrocytomas (N = 23), medulloblastomas (N = 49), ATRT (N = 9), adamantinomatous craniopharyngioma (ACP) (N = 15), papillary craniopharyngioma (PCP) (N = 3), and primary CNS lymphoma (N = 12) using tissue-microarrays and whole-slide sections (N = 577 tumors).
Results: RNA-profiling, proteomic data, and IHC each showed consistent and subtype-specific expression of ADC targets. HER3, B7-H3, and Nectin4 were expressed by most tumors from all histologic subtypes with variable intensity, including moderate-strong expression of each in GBM. Ependymomas strongly express HER2 (36/44 by IHC;82%). Meningiomas exhibited weak-moderate HER2 expression (208/248;84%). Craniopharyngiomas exhibited strong/diffuse B7-H3 (18/18;100%), and intense TROP2 in whorled epithelium in ACP (15/15;100%). ATRT strongly expressed CLDN6 (8/9;89%)
Conclusions: CNS tumors show strong and sub-type specific expression of ADC targets. These data indicate that CNS tumors may be vulnerable to multiple ADC, including those FDA-approved for other indications, and clinical trials to assess their efficacy in CNS tumors may be fruitful.

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Institutional experience of routine genomic profiling of cell-free DNA from cerebrospinal fluid across a wide range of malignancies

R Hickman[1], A Miller[2], J Jee[2], S Liu[2], E Pentsova[2], B Holle[3], D Ross[2], S Nandakumar[3], C Vanderbilt[2], S Rana[2], K Hill[3], K Chabot[3], C Hertz[2], C Campos[3], N Shukla[2], K Nafa[2], T Bale[2], M Karajannis[2], B Li[2], M Berger[2], M Ladanyi[2], I Mellinghoff[2], A Boire[2], R Brannon[2], M Arcila[2]
[1]Human Oncology and Pathogenesis Program, Sloan Kettering Institute, New York, NY, 10065; [2]Memorial Sloan Kettering Cancer Center; [3]Sloan Kettering Institute

Background: Next generation sequencing (NGS) of cell-free DNA (cfDNA) from cerebrospinal fluid (CSF) offers a non-invasive approach to identify somatic alterations in patients with central nervous system (CNS) cancers. Memorial Sloan Kettering Cancer Center uses an FDA-authorized NGS assay (MSK-IMPACT^{TM}) for sequencing cfDNA from CSF. We review the prior 3.5-year experience of this assay.
Methods: CSF samples were sequenced because of suspected/known CNS involvement by tumor. The most frequent tumor categories were lung carcinoma (n = 220), glioma (n = 161), and breast carcinoma (n = 148). Of 965 samples (720 patients), 519 passed quality control (QC) thresholds for reporting (principally $\geq$50X coverage or < 50X coverage with a detected somatic alteration). The vast majority of QC failures were due to low coverage (< 50X) and lack of a detectable genetic alteration (n = 441). Multivariate modelling predicted that higher DNA extraction yields, larger insert sizes and patients with metastatic tumors associated with passing QC thresholds.
Results: Of the reportable samples, 77% (n = 402) had detectable somatic alterations (total: 3483 mutations, 2118 copy number alterations and 154 structural variants), including actionable alterations, emerging resistance mutations and genetic profiles that allowed classification. Time-matched concordance analyses between tissue and CSF (n = 69) and between plasma and CSF (n = 51) showed a subset of alterations private to the CSF. Detection of somatic alterations in CSF portended worse overall survival than if otherwise undetected (hazard ratio: 3.6, 95% confidence interval: 2.9-4.3, P < 0.01).
Conclusions: NGS of CSF cfDNA can detect somatic alterations in CNS cancer patients and track tumor evolution. Detection of somatic alterations in CSF may be a useful prognostic biomarker for survival in cancer patients.

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Central and Extraventricular Neurocytomas : A 33-Year Experience at a Large Adult Tertiary Care Centre and Review of Molecular Characterization

A Khellaf[1], D Aissaoui[2], J Bahary[2], R Cayrol[2]
[1]CHUM, Montreal, Canada; [2]CHUM, Montreal, QC, Canada

Background: Central neurocytomas (CN) and extraventricular neurocytomas (EVN) are rare nervous system tumours of unknown etiology, classified as WHO Grade 2. These tumours typically show a neuronal immunophenotype but their exact genetic profile is not yet well characterized.
Methods: All consecutive cases from 1990 to February 2023 at a large tertiary reference centre (CHUM, Canada) were systematically retrieved and reviewed. We analyzed clinical/

neuroradiological/histopathological/outcome features in all cases. Key illustrative cases will be shown. A scoping review of published articles (English/French; no date restriction, up to Feburary 2023; PubMed/Embase) on molecular characterization and/or sequencing of either CNs/EVNs was also performed.

Results: 19 patients were identified:18 with CN and one female patient with EVN (diagnosis: age 23). CNs/EVNs accounted for < 1% of cases signed at our institution. Mean age at first pathological diagnosis of CN was 28.0 years (range 18-59) and females represented 78% (14/18) of patients. Patients most commonly presented with symptomatic hydrocephalus (11 cases). At first histopathological diagnosis, 8 CN cases showed a Ki-67 proliferation index over 4%. No IDH mutation or 1p/19q codeletion was detected in any case. Detailed molecular analysis panel (including NGS) was performed in two CN cases.Significant oncogenic single nucleotide variants were detected in one case, involving PTEN gene.Most patients with CN underwent partial resection/biopsy initially (12 cases). 8 patients with CN received adjuvant radiotherapy. 3-year recurrence-free survival One patient with CN demonstrated aggressive local recurrence 10 years post-initial diagnosis (death at 33) despite adjuvant Temozolomide. We retrieved 38 relevant published articles up to February 2023. EVNs frequently harbored FGFR1::TACC1 fusions and other FGFR1 fusions. CNs often showed DNA copy-number alterations, or MYCN/PTEN overexpression.

Conclusions: Most patients with CN/EVN demonstrated a favourable course following surgery with/without adjuvant therapy. Further multi-center studies are needed to identify and evaluate targetable genomic alterations in these tumours, and optimize therapies in higher-risk patients.

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MTAP and p16 Immunohistochemistry as Markers for CDKN2A/B Loss in Meningiomas

H Ozkizilkaya[1], H Ozkizilkaya[2], O Basit Ogunbona[2], G Toruner[3], P Aung[2], Y Esquenazi[4], F DeMonte[3], L Ballester[2]
[1]The University of Texas MD Anderson Cancer Center Translational Molecular Pathology Department; [2]The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [3]The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [4]The University of Texas Health Science Center at Houston, Vivian L. Smith Department of Neurosurgery, Houston, TX, USA. Center for Precision Health, School of Biomedical Informatics, The University of Texas Health Science Center at Houston, Houston, TX, USA. Memorial Hermann Hospital-TMC, Houston, TX, USA

Background: CDKN2A encodes for p16, a cell-cycle regulator. In the 2021 WHO classification of CNS tumors, CDKN2A/B loss is included as criteria for WHO grade 3 meningiomas. CDKN2A/B loss can be detected by NGS or FISH. However, these techniques are costly and not always readily accessible. An immunohistochemistry-based biomarker for evaluating CDKN2A/B loss in meningiomas would provide faster results at a lower cost. T MTAP gene is in close physical proximity to CDKN2A/B in chromosome 9 and deletions in this region frequently involve both CDKN2A/B and MTAP.

Methods: Fifty meningiomas (grade 1 (n = 26), grade 2 (n = 16), and grade 3 (n = 8)) were included in the study. Demographic, histologic and survival data were obtained from electronic medical records. Tissue Microarrays were constructed using representative tumor areas for each patient. H&E stains and immunohistochemistry (IHC) for MTAP and p16 were performed and evaluated by a board-certified neuropathologist as positive or negative. FISH for CDKN2A was chosen as the gold standard to assess the sensitivity and specificity of MTAP and p16 staining as surrogate markers of CDKN2A/B loss.

Results: A total of 49 tumors were available to evaluate MTAP expression. Forty-six cores (94%) showed strong MTAP staining, and FISH confirmed intact CDKN2A status. One meningioma grade 3 was MTAP negative and FISH demonstrated homozygous CDKN2A loss. Two grade 2 meningiomas showed focal MTAP expression, one of these cases showed regional CDKN2A loss by FISH. The sensitivity of MTAP for identifying meningiomas with CDKN2A loss was100% (2/2). The specificity of MTAP expression for identifying intact CDKN2A status was 98% (46/47). P16 expression was variable and did not correlate with either MTAP IHC or CDKN2A FISH results.

Conclusions: MTAP is a promising surrogate marker for CDKN2A homozygous loss in meningiomas. Tumors with focal MTAP expression should be further investigated with FISH to confirm CDKN2A homozygous loss.

PLATFORM 7: New Methods/Technologies and Other

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Combining copy number and methylation data from DNA methylation arrays can improve classification and risk stratification models

B Orr[1], M Alom[2], Q Tran[2]
[1]St. Jude Children's Research Hospital; [2]St. Jude Children's Research Hospital

Background: DNA methylation arrays are an important tool for clinical classification of brain tumors. Methylation-based classification models are trained on the differentially methylated probes from a reference series of tumors, and then applied to diagnostic test samples. Although copy number profiles are routinely captured from array data, copy number abnormalities are reported independent of classification models, and multimodality models have not been reported. Individual tumor classes may be associated with specific clinical risk, but current models do not provide a within-class risk, comparable to histologic grading.

Methods: To evaluate the utility of combined multimodality methylation and copy number data for computational modeling, we established a workflow to uniformly extract copy number features from the output of the array-based 'conumee'

copy number algorithm. Using a cohort of diffuse gliomas from The Cancer Genome Atlas, we trained deep neural net models on two tasks: tumor classification and survival prediction, using either methylation-data alone, copy number data-alone, or combined copy number and methylation-data. The classification models were compared in cross validation and holdout test sets for accuracy, precision, recall, and F1 score. Survival models were compared using the c-index (CI).

Results: We find that training on copy number-alone is inferior to methylation-alone or multimodality data for tumor subclass classification (84.7 % top accuracy, compared to 95.1 % and 95.8 % respectively). In survival analysis, multimodality modeling improved survival prediction compared to DNA methylation and copy number-alone (CI = 86.2, compared to 83.5 and 82.2, respectively).

Conclusions: We demonstrate a novel method to systematically integrate copy number data from methylation arrays into clinically relevant computational models and find multimodality models improve the performance of both classification and outcome prediction. Our method for survival prediction is generalizable and, with appropriate training data, could be used to perform molecular grading within other tumor classes.

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Whole Genome HiC Identifies Structural Rearrangements in Primary CNS Lymphomas

M Movahed-Ezazi[1], K Galbraith[1], I Tran[1], H Mohamed[1], Y Yang[1], A Arbini[1], N Ward[1], L Chiriboga[1], K Sikkink[2], A Schmitt[3], M Snuderl[1]
[1]NYU Langone Health; [2]Arima Genomics; [3]Armina Genomics

Background: CNS lymphomas frequently contain gene rearrangements that are usually probed using fluorescence in-situ hybridization. Karyotyping is rarely possible in CNS lymphomas and therefore complex rearrangements often remain undetected. Whole genome Hi-C is a DNA-based assay that combines proximity-based ligation with massive parallel sequencing, and can detect gene rearrangements and also analyzes 3D genomic architecture.

Methods: FFPE tissue from 8 primary CNS lymphomas were subjected to Hi-C using Arima HiC+ for FFPE kit. After Hi-C and DNA purification, libraries were prepared for paired-end Illumina sequencing, and sequenced to an average of 10X genome coverage on NovaSeq. Data was analyzed using the Arima-SV pipeline using Juicer and HiCUP, gene rearrangement detection using HiC-Breakfinder, loop calling using Juicer Tools, and integrative data visualization using Juicebox. Overexpression of putative driver genes was confirmed by immunohistochemistry (IHC).

Results: Hi-C analysis revealed specific rearrangements in six out of eight cases studied. In two cases, we identified rearrangements between BCL6 and promoters of IgH and IgL genes, respectively, and in one case a fusion between MYC and IgH promoter. In a case of presumed classic Hodgkin's lymphoma, we identified a PTPRD rearrangement, usually seen in nodal marginal zone lymphomas. Two CNS lymphomas showed complex genomes with numerous rearrangements. One with RET, RAD51C, and TP63 rearrangements, and the other with PD-L1, ROS1, JAK2, RAF1 rearrangements. All detectable targets were confirmed by IHC. Two CNS lymphomas showed no detectable rearrangements.

Conclusions: Whole genome Hi-C successfully identifies a spectrum of complex structural rearrangements and provides deeper insight in molecular drivers of primary CNS lymphomas. Hi-C also identifies rearrangements between promoters and coding regions undetectable by RNA-based next-generation sequencing. Our study shows the advantage of 3D genomics approach for detecting structural rearrangements that may be missed using the standard method of FISH or cytogenetic studies providing diagnostic and therapeutic targets.

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Rapid assessment of neuropathology specimens using nonlinear microscopy

H Varma[1], T Weber[2], J Fujimoto[2], S Rosen[3]
[1]Beth Israel Deaconess Medical Center; [2]MIT, Cambridge, MA; [3]Beth Israel Deaconess Medical Center, Harvard Medical School

Background: Histological processing for microscopic evaluation of pathological tissue specimens is a time and resource consuming multi-step process. New technologies for rapid tissue imaging that involve less processing and are amenable histological interpretation similar to formalin fixed, paraffin embedded sections would greatly alleviate this bottleneck.

Methods: We have developed and applied a laser-scanning imaging technique, nonlinear microscopy (NLM), to visualize isolated optical sections with depths up to 100 microns of surface of intact, non-processed neural tissue. Tissue specimens are rapidly stained with fluorescent nuclear and stromal dyes, and nonlinearly excited by a scanned focused femtosecond laser. The fluorescent signals are digitally recolored to closely resemble conventional H&E-stained sections. We imaged different parts of brain, in fresh and fixed un-sectioned tissue using real time NLM.

Results: This assessment revealed robust identification of diverse cell-types including: neurons, glia, endothelium, and axons. Analysis of representative neuropathological specimens from surgical and autopsy cases with varied pathologies including neoplastic, vascular, inflammatory and neurodegenerative disorders readily identified diagnostic features such as "red" neurons, macrophages, amyloid plaques, tangles, necrosis, nuclear atypia, mitoses and vascular proliferation.

Conclusions: This work suggests the feasibility of NLM for rapid diagnosis in neuropathology settings and lays the foundation for future studies to evaluate use in routine pathology practice. Other applications include teaching, research and

forensic neuropathology where rapid microscopic histological examination can supplement gross examination. Supported in part by NIH Grant RO1 CA249151-03

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Feasibility of self-supervised learning for diagnosing Alzheimer's disease and tauopathies

S Koga[1], B Jo[2], M Kim[2], D Ono[1], H Sekiya[1], D Dickson[1], T Hwang[2,3,4]
[1]Department of Neuroscience, Mayo Clinic, Jacksonville, Florida, USA; [2]Department of Artificial Intelligence and Informatics Research, Mayo Clinic, Jacksonville, Florida, USA; [3]Department of Cancer Biology, Mayo Clinic, Jacksonville, Florida, USA; [4]Department of Immunology, Mayo Clinic, Jacksonville, Florida, USA

Background: Pre-training models on ImageNet, which comprises a diverse range of images, has been widely utilized in machine learning in medical imaging; however, fundamental differences exist between generic images from ImageNet and histology images. This study explores different deep learning approaches to developing a diagnostic model for Alzheimer's disease and tauopathies using a limited number of whole slide images (WSIs); a self-supervised learning (SSL) model pre-trained on brain histopathology images, and a pre-trained model on ImageNet.
Methods: The SSL model was pre-trained on 600 WSIs of H&E and immunohistochemistry (tau and α-synuclein) from various brain regions. For disease classification, the corpus striatum section of patients with AD (n = 30), corticobasal degeneration (n = 20), globular glial tauopathy (n = 10), Pick's disease (n = 20), and progressive supranuclear palsy (n = 20), as well as non-tauopathy controls (n = 21) were immunostained with phosphorylated-tau and scanned to WSIs. We then applied deep learning models (CLAM) to the WSIs.
Results: The SSL-pretrained model outperforms the others with higher area under the curve (AUC: 0.979 ± 0.031) and accuracy (83.3%), compared to the ImageNet-pretrained model (AUC: 0.962 ± 0.029; accuracy 79.2%) for all training datasets, as well as with smaller datasets. The difference in performance between the two models is more pronounced with fewer samples; AUC of 0.902 ± 0.067 for the SSL model and 0.931 ± 0.077 for the ImageNet-based model, when trained with only one sample per class.
Conclusions: We show that SSL pre-training with brain histology images surpasses ImageNet pre-training with high AUC (0.979) and accuracy (83.3%) in diagnosing five tauopathies. The SSL-pretrained model is robust even when using extremely limited datasets. We achieve superior AUC and accuracy, demonstrating the feasibility of establishing a reliable diagnostic model for AD and tauopathies with limited labeled data. This approach can be applied to rare neurodegenerative diseases with few cases available.

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Scalable Deep Learning Workflows for Quantifying Neurofibrillary Tangles in Human Brain Samples

S Ghandian[1], K Nava[2], L Minaud[1], L Beckett[3], R Rissman[4], A Teich[5], B Dugger[6], M Keiser[1]
[1]Institute for Neurodegenerative Diseases, University of California, San Francisco, San Francisco, CA, 94158, USA; [2]Department of Pathology and Laboratory Medicine, School of Medicine, University of California, Davis, Sacramento, CA 95817, USA; [3]Department of Public Health Sciences, School of Medicine, University of California, Davis, Sacramento, CA 95817, USA; [4]Department of Neurosciences, University of California San Diego, San Diego, La Jolla, California; [5]Taub Institute for Research on Alzheimer's Disease and Aging Brain, Department of Neurology, Department of Pathology and Cell Biology, Columbia University Medical Center, New York, New York; [6]Alzheimer's Disease Research Center, Department of Neurology, University of California Davis, School of Medicine, Sacramento, California

Background: Accumulation of abnormal tau protein as neurofibrillary tangles (NFTs) is a pathologic hallmark of Alzheimer's disease. Accurate and efficient detection and quantification of NFTs in tissue samples can aid in deeper phenotyping of Alzheimer's disease and may reveal additional relationships with clinical, demographic, and genetic features. Currently, expert manual analysis can be time-consuming, subject to observer variability, and limited in handling the large amounts of data generated by modern imaging techniques.
Methods: We present a scalable deep learning-based approach to quantify neurofibrillary tangle (NFTs) burden in digital whole slide images (WSIs) of post-mortem human brain tissue. Our approach uses a UNet model trained on 45 annotated regions of interest selected from 15 unique WSIs of temporal cortex from Alzheimer's disease cases from three institutes (University of California (UC)-Davis, UC-San Diego, and Columbia University). We developed a custom method to generate segmentation ground truth masks from point annotations of three 2400μm by 1200μm regions of interest per WSI.
Results: The model achieved a precision of 0.73, recall of 0.68, and F1 score of 0.70 on a held-out test set of 7 WSIs, providing neuropathologists with an efficient and reliable tool for tau burden quantification.
Conclusions: Our approach offers a proof of concept to enable a detailed and scalable analysis of large cohorts, which is not feasible through manual assessment. Visualizing the spatial

distribution and morphology of NFTs in tissue through WSI-level segmentation heatmaps is also a significant advantage, offering a more complete understanding of disease progression.

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Crowd powered neuropathology annotations for machine learning algorithms: A Citizen Science pilot study focused on the Hispanic community

B Dugger[1], P Michelucci[2], L Gusman[2], P Santander[2], M Lane[2], R Dussaruth[2], G Vaičaitytė[2], J Lou[3], S Magaki[4], L Minaud[5], M Keiser[5]
[1]University of California, Davis; [2]Human Computation Institute; [3]University of California, Irvine; [4]University of California, Los Angeles; [5]University of California, San Francisco

Background: There is a need to provide more objective finer grained pathological evaluations in a scalable manner as well as increase community outreach and education especially within the Hispanic community. To fill these gaps, we piloted a citizen science project involving new human computation methods to engage public volunteers in the histopathological analysis of whole slide images (WSI) of human brain. Citizen science is the practice of public participation and collaboration in research to increase scientific knowledge.
Methods: Our pilot study utilized a crowd-powered analytic pipeline breaking down histopathological analysis into a stepwise series of manageable online tasks that can be accomplished by non-expert members of the general public. We also incorporated "wisdom of crowd" methods for each step of the analysis that combine individual answers into a single expert-like answer. This pilot compared the two-stage crowd-based analysis to gold standard data generated by neuropathology experts for two major pathology categories: cored plaques and cerebral amyloid angiopathy.
Results: Through methodological development, including testing of annotation interfaces and consensus methods, we found with sufficient aggregation of non-expert answers, it is possible to consistently achieve a level of agreement with gold standard data equivalent to interrater agreement among expert annotators.
Conclusions: These approaches support the rapid production of high-volume datasets for use in machine learning algorithm development to provide deeper phenotyping of neurodegenerative diseases. These pilot results support the plausibility of moving forward with an openly available online platform, which will be used to execute the first ever de-novo analysis of Alzheimer's histopathology imagery by a crowd of volunteers (geared towards those of Hispanic heritage). The platform will engage Hispanic communities directly impacted by this research, while providing a culturally responsive outreach and inclusion program for these communities to represent themselves in discussions with researchers.

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Applying Neural Networks to Neuropathology: Review of Two Artificial Intelligence Chatbots

R Hickman[1], C Vanderbilt[2], D Priemer[3], R Folkerth[4], D Perl[5]
[1]Murtha Cancer Center Research Program, USUHS, Bethesda, MD, USA; [2]Memorial Sloan Kettering Cancer Center; [3]Uniformed Services University of Health Sciences; [4]New York University Grossman School of Medicine; [5]Uniformed Services University of the Health Sciences

Background: ChatGPT and ChatSonic are artificial intelligence (AI) models trained to generate human-like responses using vast amounts of text. The growing popularity of AI technology has garnered interest about its capabilities but also concern regarding misuse in academic and scientific settings. We assessed the proficiency of ChatGPT and ChatSonic as applied to neuropathology.
Methods: A battery of 50 multiple-choice questions (MCQs) and 10 open-ended questions aligned to the 2022 American Board of Pathology Blueprint for Neuropathology was administered to both models. They were also tasked with generating templates for pathology reports (n = 3), correspondences to colleagues (n = 3), and programming scripts (n = 3). The models' responses were compared to ground truth.
Results: ChatGPT outperformed ChatSonic with MCQs (64% vs. 50%). Frequent errors related to different eponym uses in neuropathology (e.g. Creutzfeldt cell versus Creutzfeldt-Jakob Disease) or choices where a vignette had overlapping characteristics with various answer options. ChatGPT and ChatSonic correctly answered 60% and 50% of open-ended questions, respectively; ChatGPT produced qualitatively better answers than ChatSonic. ChatSonic frequently issued citations to support its responses; ChatGPT incorrectly generated several citations. ChatGPT provided the best templates, but regardless, both models necessitate close review by the user to detect errors. Both models delivered useful coding examples; ChatGPT was preferred.
Conclusions: Overall, ChatGPT surpassed ChatSonic. While both models have potential to assist pathologists in their daily work, particularly in generating drafts for correspondence and coding templates, the error rate to neuropathology questions was considered too high to be reliable. Until improved, these models should be used with caution and only after close review of their output. Disclaimer: The information/content and conclusions do not necessarily represent the official position or policy of, nor should any official endorsement be inferred on the part of the Department of Defense, US Government or the

Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc.

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Diversify In Pathology: Leveraging Podcasting to Address Diversity, Equity and Inclusion in Pathology

M Williams[1], R Balassanian[2], C Parra-Herran[3]
[1]UAB Department of Pathology; [2]University of California, San Francisco; [3]Brigham and Woman's Hospital Harvard Medical School

Background: The Diversify in Pathology (DIP) podcast (https://www.diversifyinpathpodcast.com/) was created in August 2021 to explore issues of diversity, equity, and inclusion (DEI) in pathology. Through online tools and recording equipment, successful interviews with notable and emerging people in the pathology community have been shared, including their unique perspectives on DEI issues. We measured the impact of the DIP Podcast on the pathology community.
Methods: A total of 36 speakers were invited and interviewed by the host (MW) over one year. The interviews were broadcast via various podcast platforms and shared with the pathology community. Engagement with DIP was measured through the analytics provided by the podcast-hosting website Buzzsprout and the social media platform Twitter. Demographics were collected from speakers during the interview process
Results: Between August 1st, 2021, and July 1st, 2022, corresponding to seasons 1 and 2 of the DIP podcast, we recorded 3,700 downloads, equating to an average of 300 episode downloads per month, directly gauging listener engagement. Most downloads were within North America (87%). Over seven months, the monthly average impressions and engagements on Twitter were 7,662 and 99, respectively. Via self-identification, the speaker pool included the following groups: 19 (53%) women, 17 (47%) men, 17 (47%) Black, 7(19%) Whites, 3 (8%) Asian American, 5 (14%) Latinx and 5 (14%) LGBTQ+.
Conclusions: Podcasting is an effective platform for asynchronous learning in medical education and diversity, equity, and inclusion (DEI) efforts. The DIP Podcast has generated engagement from a diverse pool of representatives of the pathology community and has reached a significant number of listeners in North America and worldwide. DIP has increased awareness and discussion around DEI issues in Pathology and Laboratory Medicine.

PLATFORM 8: Infectious, Demyelinating, and Inflammatory

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CSF Immune Factor-Based Extreme Gradient Boosting Algorithm Helps Distinguishes Infectious and Non-infectious CNS Disorders

T Chao[1], R Kean[1], A Cárdenas[2], M Pettengill[1], J Nassur[1], A Viaene[2], D Hooper[1], M Curtis[1]
[1]Thomas Jefferson University, Philadelphia, PA, USA; [2]Children's Hospital of Philadelphia

Background: Rapid diagnosis is critical to therapeutic outcomes for many CNS diseases, especially infections. The application of machine learning to identify disease-specific CSF immune factor profiles may aid in the use of such information to rapidly distinguish CNS disorders. Here we report the use of Extreme Gradient Boosting (XGB) machine learning algorithm to classify patient CNS disorder based on CSF immune factors.
Methods: We analyzed 102 CSF samples from patients with CNS diseases and from patients with no identified CNS pathology (controls). The median fluorescent intensity (MFI) and factor concentrations for 13 complement factors and 41 cytokines were determined using a fluorescent microsphere based multiplex system. The samples were randomly split into a training set (n = 82: 23 controls, 25 viral, 14 bacterial, 10 tumor, 10 autoimmune/inflammatory) and a testing set (n = 20: 6 controls, 6 viral, 4 bacterial, 2 tumor, 2 autoimmune/inflammatory).
Results: In the training set, statistically significant variations were observed for 47 of the 54 CSF factors using the Kruskal-Wallis test. Linear discriminant analysis (LDA) and hierarchal clustering of the training set data were both able to cluster CNS disease states. An XGB model was built to classify the CNS disease state of each training set sample using all 54 CSF factors. SHapley Additive exPlanations (SHAP) values were used to rank the importance of each analyte in the model. Complement factor C2 was identified as the most important factor. Also identified were analytes most informative for each group (control: low C2; bacterial infection: high IL-17a; viral infection: high IP-10; tumor: low IL12p70; autoimmune/inflammation: low IL-7). The XGB model was next applied to the test samples and achieved an overall weighted accuracy of 65% (controls: 67%; bacterial infections: 75%; viral infections: 83%; autoimmune/inflammation: 50%; tumors: 0%).
Conclusions: Our work highlights the potential for CSF immune factor-based algorithms to distinguish different classes of CNS disorders.

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Neuropathology of First Human Rabies Virus Encephalitis Case Treated with Favipiravir and AAV-RAB

R Folkerth[1], S Thomas[2], N Boire[3], K Cervellione[4], A Davis[5], C Epstein[6], S Munjal[7], K Van Kampen[8], M Sena-Esteves[9], D Martin[10], H Baker[10], R Willoughby[11], I Solomon[12], D Priemer[13], D Perl[14]
[1]New York City Office of Chief Medical Examiner; [2]Office of the District 21 Medical Examiner, Fort Myers FL; [3]Division of Anatomical and Clinical Pathology, Mayo Clinic, Rochester MN; [4]Flushing Hospital Medical Center, New York NY; [5]Griffin Laboratory, New York State Department of Health, Wadsworth Center, New York NY; [6]Fujifilm Pharmaceuticals, USA; [7]Dr. Reddy's Laboratories, Princeton, NJ; [8]Van Kampen Group, Payson UT; [9]Horae Gene Therapy Center, University

of Massachusetts Medical School, Worcester MA; [10]Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, Auburn, AL; [11]Medical College of Wisconsin, Milwaukee WI; [12]Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston MA; [13]Department of Pathology, F. Edward Hébert School of Medicine, Uniformed Services University, Bethesda MD; Department of Defense/Uniformed Services University Brain Tissue Repository, Uniformed Services University, Bethesda MD; The Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda MD; [14]Department of Pathology, F. Edward Hébert School of Medicine, Uniformed Services University, Bethesda MD; Department of Defense/Uniformed Services University Brain Tissue Repository, Uniformed Services University, Bethesda MD

Background: Although rabies encephalitis is among the most rapidly lethal zoonoses, postmortem analysis remains uncommon, due to effective pet vaccination as well as pre- and early post-exposure prophylaxis (PEP) to prevent symptomatic disease. Even more rare is analysis of symptomatic cases treated with novel experimental therapies, with survivals beyond a few days.
Methods: We present a case and review the neuropathology, placed in historical perspective, of a 59-year-old man with 2 days of nausea, who became rapidly encephalopathic. Six months earlier, he had been bitten by a dog while in the Philippines but did not receive PEP. PCR from saliva was positive for rabies (Philippines dog variant), and nuchal biopsy was antigen-positive. The Milwaukee protocol (intensive general medical support) was initiated. Through compassionate use protocols, favipiravir (anti-rhabdoviral) and adeno-associated virus gene therapy expressing monoclonal antibody (AAV-RAB) were given on Days 7 and 9, respectively. Although AAV-RAB showed immediate, self-limited evidence of transduction in the liver, with serum and CSF neutralizing antibody detection on Days 13 and 20, respectively, and salivary clearance of virus on day 20, the patient worsened clinically and died on Day 28.
Results: Brain-only autopsy detected cultivable virus, with neuronal loss accompanied by marked lymphoplasmacytic and microglial inflammation, including Babes' nodules, in essentially all cerebral gray matter. Inflammation was prominent in the leptomeninges, neurohypophysis and oculomotor nerve. Ischemic changes were widespread. The degree of inflammation in the current case was far in excess of that seen in personal experience and published historical analyses by one of us (DPP).
Conclusions: Novel use of favipiravir and AAV-RAB in a man with rabies encephalitis resulted in some clinical laboratory parameters of response, however, post-mortem neuropathology revealed an unusually robust inflammatory response, of uncertain relationship to the therapy, to the prolonged clinical course (not typical in historic cases), or both.

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Postmortem Assessment of Olfactory Axon Degeneration and Microvasculopathy Associated. with SARS-CoV-2 Variants

C Ho[1], T Zaikos[1], M Salimian[2], M Morris[1], H Ames[3], C Drachenberg[3], J Papadimitriou[3], J Hegert[2]
[1]Johns Hopkins University School of Medicine; [2]Orlando Health; [3]University of Maryland School of Medicine

Background: Olfactory dysfunction is a common presentation of COVID-19. Viral variants have been associated with differing incidences of olfactory dysfunction. We have previously demonstrated that COVID-19 is associated with axon and microvascular injury in olfactory bulb and tract. Here, we aim to determine whether trends in olfactory dysfunction associated with SARS-CoV-2 variants result from differences in olfactory axon and microvascular pathology.
Methods: We conducted a multi-institution, post-mortem cohort study. Olfactory bulb and tract tissue was collected from deceased patients who had PCR-confirmed COVID-19 or from appropriate controls. Axon and microvascular pathology of olfactory tissue was evaluated by electron microscopy. Axon density was calculated using ImageJ. Preliminary SARS-CoV-2 variant identity was inferred by comparing dominant viral variants at the time of SARS-CoV-2 infection.
Results: Olfactory tissue was collected from 32 deceased patients with COVID-19 [median age, 65; 22 men (69%)] and from 16 controls [median age, 53.5; 9 men (56%)]. There were 10 (31%), 2 (6%), 12 (38%), and 8 (25%) infections by the 19A-20G, Alpha, Delta, and Omicron variants, respectively. Alpha cases were excluded from statistical analyses. Olfactory dysfunction was reported in 4 (40%), 5 (42%), and 0 cases of 19A-20G, Delta, and Omicron variant infection, respectively. Olfactory tissue axon and microvascular pathology was significantly greater among all variants of COVID-19, as compared to olfactory tissue from control cases. Although not statistically significant, trends towards higher axon and microvascular pathology scores among those cases with olfactory dysfunction were observed. There was no obvious difference in axon or microvascular pathology between different variants.
Conclusions: Infection by SARS-CoV-2 variants is associated with a variable incidence of olfactory dysfunction. While overall there is no obvious difference in olfactory pathology between different virus variants, it is possible that our sample represen-

tation skews towards cases with intact smell. Further analysis with more samples is underway to address this issue.

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Neuropathologic comorbidities in longstanding HIV infection: An autopsy study

S Magaki[1], J Gonzalez[2], N Nemanim[2], N Rahimzadeh[2], A Murillo[2], R Diaz[2], J Morales[2], D De Los Santos [2], J Said[2], N Khanlou[2], W Yong[2], H Vinters[2], E Singer[2]
[1]Section of Neuropathology, Department of Pathology and Laboratory Medicine, Ronald Reagan UCLA Medical Center and David Geffen School of Medicine; [2]Ronald Reagan UCLA Medical Center and David Geffen School of Medicine

Background: HIV infection is associated with a wide range of neurologic comorbidities. The success of combined antiretroviral treatment has extended the life expectancy of people living with HIV to approximate that of the general population, which has increased the age of the cohort as well as duration of HIV infection. In this study, we examined autopsy brains from HIV-positive subjects with varying duration of HIV infection to characterize neuropathologic findings in longstanding HIV infection.
Methods: We examined the brains of 229 HIV-positive subjects enrolled in the National Neurological AIDS Bank, part of the National NeuroAIDS Tissue Consortium, which follows HIV-infected individuals, many of whom have undergone extensive neurocognitive and laboratory testing, to autopsy. This HIV-positive cohort was grouped according to length of HIV infection: >20 years (n = 47, mean age 54.4 ± 10.9 years, M:F = 15), 10-19 years (n = 95, mean age 50.3 ± 9.6 years, M:F = 6), and < 10 years (n = 87, mean age 45.9 ± 12.4 years, M:F = 8).
Results: The groups were significantly different in age. A similar percentage of subjects (6-10%) in each group demonstrated HIV encephalitis. CMV encephalitis (4 subjects) and active Toxoplasmosis (2 subjects) were only observed in the group with < 10 years of HIV infection. Primary CNS lymphoma was seen in 8 subjects, 6 of whom had < 10 years of HIV infection. A higher proportion of subjects with >20 years of HIV infection showed ischemic lesions.
Conclusions: While rates of HIV encephalitis were similar across groups, active opportunistic infections and primary CNS lymphoma were more common in subjects with shorter duration of HIV infection. Ischemic changes were more common in subjects with longer duration of HIV infection. Understanding the neuropathologic comorbidities in patients with longstanding HIV infection is important in the care of these individuals.

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Neuropathological Findings in Immune-Mediated Encephalitides: An Autopsy Series

E Conner, M Martinez-Lage
Massachusetts General Hospital

Background: Immune-mediated encephalitides are inflammatory disorders of the central nervous system associated with malignancy (paraneoplastic) and/or with autoantibodies against intracellular or surface neuronal antigens (autoimmune). Comprehensive neuropathological descriptions of these disorders are sparse. We aim to describe the findings in autopsy cases of immune-mediated encephalitides.
Methods: Autopsy report archives of the Massachusetts General Hospital from 2000-2022 were used to identify cases, using the search terms "encephalitis", "paraneoplastic", and "antibody". Cases with a confirmed or suspected infectious etiology were excluded.
Results: 18 cases met criteria, with an age range of 32-91 (average 69); 66.6% were male. There were 10 cases of paraneoplastic encephalitis, with small cell lung carcinoma being the most common malignancy (4) followed by thymoma (2), lymphoma (2), serous carcinoma (1), and pancreatic adenocarcinoma (1). 3 of these 10 cases presented with specific antibodies: LGI-1, ANNA-1 (anti-Hu), and PCA1 (anti-Yo). In one case immunotherapy-related encephalitis was suspected. Lymphocytic infiltrates with perivascular cuffing were the most common histologic finding (89%), associated with microglial nodules in 50% of cases, and with neuronal loss and gliosis of involved sites in 78% of cases. Structurally, most cases involved the cerebrum (82%), most commonly localizing to the hippocampus, echoing the common syndrome of limbic encephalitis. Inflammatory leptomeningeal involvement/meningitis was present in 41% of cases. Brainstem involvement was common (59%), while involvement of the spinal cord was rare (12%).
Conclusions: Neuropathological findings in immune-mediated encephalitis include active lymphocyte-rich inflammation and neuronal loss, localize to regions clinically involved and beyond, and often include severe neuronal loss of target structures such as the hippocampal formation and the cerebellum. Specific autoantibodies are identified in recent cases, highlighting our progressive understanding of the underlying pathophysiology of these disorders.

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Digital Spatial Profiling of Gene Expression in Murine Models of Age-Related Neurodegeneration

C Wiley[1], R Acharya[1], R Bao[2], B Isett[1], J Kofler[1], D Marker[3], E Meyer[1], A Signore[1], Q Wang[1]
[1]University of Pittsburgh; [2]University of Pittsburgh; [3]University of Pittsburgh

Background: Previously we presented murine models of the human neurodegenerative disease Aicardi-Gutiérrez Syndrome. Mice carrying a homozygous mutation in adenosine deaminase acting on RNA gene (ADAR1) demonstrated a deficit in RNA editing that lead to MDA-5 mediated RNA sensing pathway activation of the innate immune response (IIR). Despite months of elevated interferon stimulated gene (ISG) expression, there was essentially no inflammatory infiltrate and limited neuropathology of deep gray matter mineralization developing between 8 and 12 months of age

Methods: To test whether beta amyloid accumulation would trigger a classical inflammatory profile in the mutant mice, we crossed the ADAR1 mutants with mice carrying mutations in amyloid precursor protein and presenilin genes.
Results: At 5 months of age early beta amyloid deposits were detected in both AD and ADAR1/AD mice. However, AD mice showed no augmented expression of IIR genes and ADAR1/AD mice showed similar IIR expression to that seen in mice carrying the ADAR1 mutation alone. Next, we examined whether Digital Spatial Profiling (DSP) of the whole murine transcriptome (WTA) could assess regional and temporal differences in gene expression of WT and ADAR1 mutant mice.
Conclusions: Confirming the validity of DSP, WTA of brain regions that showed elevated expression of ISG-15 or CXCL-10 by in situ hybridization, demonstrated elevation of other innate immune response genes previously shown in RNA extracted from whole brain homogenates. The WTA permitted an unbiased assessment of all altered gene expression in brain regions differentially expressing ISG-15. Thus, DSP permits unbiased WTA in different brain regions throughout the time course of neurodegeneration and will be useful in discovering novel degenerative molecular pathways. Differences in gene expression in the near absence of neuropathology highlights limitations of morphological analysis of neurodegeneration.

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Evidence that T-cell targeting of specific brain nuclei as a frequent pathogenic mechanism of human obesity

J Ahrendsen[1], Y Nong[2], Y Huo[2], J Steele[3], M Anderson[4]
[1]Northwestern University Feinberg School of Medicine; [2]Regeneron Pharmaceuticals; [3]Beth Israel Deaconess Medical Center; [4]Regeneron Pharmaceuticals and Beth Israel Deaconess Medical Center

Background: Obesity, defined as dysregulation of energy balance, causes significant morbidity in affected individuals and results in substantial healthcare-related expenditures worldwide. The pathogenesis and pathophysiology underlying obesity is complex, with influences from the environment, genetic susceptibility, individual behavior, and co-morbid conditions. The hypothalamus is a key central regulator of several homeostatic functions, including feeding behavior and energy expenditure, and accumulating evidence implicates hypothalamic dysfunction in generating an obese phenotype.
Methods: We performed a thorough and systematic analysis of lymphocyte-related inflammation in a large cohort of human post-mortem brains from obese ($n = 25$) and non-obese ($n = 25$) decedents, examining numerous brain regions in addition to the hypothalamus. We also utilize a mouse model in which an immunogenic adeno-associated virus (AAV) virus expressing GFP peptides under a general CMV promoter was injected in the ventral medial hypothalamus region in order to recapitulate the pathology observed in the brains of human decedents with obesity.
Results: We show that in obese patients, T-cells are significantly increased in the median eminence/arcuate nucleus (ME/Arc) of the hypothalamus compared to non-obese patients. Elevated inflammation was specific to the ME/Arc, and not present in other hypothalamic nuclei or other distant brain regions, and was composed primarily of CD8-positive cytotoxic T-cells. We also show that there is evidence of neuronal damage in the ME/Arc of obese patients in association with the T-cell inflammatory infiltrates. Finally, we show that in mice injected with AAV-CMV-GFP into the ventral medial hypothalamus, a localized T-cell inflammatory response is generated and the animals develop an obese phenotype, neither of which were present in saline injected control animals, thereby recapitulating the pathology observed in human post-mortem brains.
Conclusions: This study identifies T-cell immune responses in specific hypothalamic brain regions associated with obesity, implicating a potential new therapeutic target in human patients.

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A natural model of multiple sclerosis to assess novel therapeutics targeting the neuropathology associated with disease progression

M Church[1], U Boschert[2], C Vite[1], J Alvarez[1]
[1]University of Pennsylvania, School of Veterinary Medicine; [2]Ares Trading SA, an affiliate of Merck KGaA

Background: Multiple sclerosis (MS) is a chronic neuroinflammatory disease affecting over 1.5 million people in the US. Most patients initially experience relapsing remitting MS and subsequently progressive MS (PMS). Neurodegenerative changes in PMS correlate with compartmentalized neuroinflammation characterized by B-cell rich meningeal infiltrates and neighboring activated microglia. PMS remains challenging to treat due to the inability of rodent models to replicate the neuropathology thought to drive disease progression. Thus, identifying models with similar pathologic features of PMS is urgently needed. We have demonstrated that granulomatous meningoencephalomyelitis (GME), a neuroinflammatory disease in dogs with epidemiological resemblance to MS, exhibits compartmentalized neuroinflammation that correlates with submeningeal cortical demyelination as in PMS.
Methods: To strengthen this natural model, we investigated the immune response in CSF and GME tissues via flow cytometry and immunostaining. We evaluated BTK as a potential therapeutic target in GME utilizing immunostaining.
Results: We found prominent B cell accumulation and robust antibody production in CSF. Importantly, B cells are an attractive therapeutic target in MS, though CNS B cells are protected from most antibody-based therapies. An alternative approach is targeting CNS inflammation by inhibiting Bruton's tyrosine kinase (BTK), an enzyme regulating B cell activation and survival, but also expressed by macrophages and microglia.

BTK inhibitors are small molecules able to enter the CNS. We found marked BTK expression within B cell-rich leptomeningeal infiltrates, in B cells extending into Virchow Robin spaces, and in perivascular cells moving into the parenchymal tissue. Additionally, BTK was detected in microglia/macrophages in leptomeningeal infiltrates and in the adjacent parenchyma.
Conclusions: Beyond mechanistic studies, this natural model would deliver immediate translational benefits given the 1) similar clinical metrics to assess disease progression and response to therapy in dogs and humans and 2) ease of application of findings, given the use of dogs for final drug assessments prior to human use.

SATURDAY POSTERS: Tumors: Nonglial

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Giant Epidermoid Cyst of Posterior Fossa

H Dai[1], A Carter[1], M Karsy[2], S Menacho[3], Q Mao[3], L Robinson[3]
[1]Eastern Virginia Medical School; [2]Drexel University, Philadelphia, PA, USA; [3]University of Utah, Salt Lake City, Utah, USA

Background: Epidermoid cysts are slow-growing benign neoplasms that account for 1% of all brain tumors. Cysts that are 5cm or greater in size are commonly known as giant epidermoids and are typically seen in patients aged 30-50. Due to their slow-growing nature, giant epidermoid cysts often have insidious clinical onset in the form of vascular or neurological disturbances. Though commonly found in the cerebellopontine (CP) angle, epidermoid cysts presenting in the posterior fossa but outside of the cerebellopontine angle are exceedingly rare. As of 2021 and to the best of our knowledge, only 12 have been reported in the literature.
Methods: Here, we present a 67-year-old male who presented with obstructive hydrocephalus, dizziness, and a ground-level fall. His medical history is largely unremarkable except for a remote history of prior head trauma. Imaging demonstrated an 8x4 cm left cerebellar epidermoid lesion. Surgical resection utilizing a lateral suboccipital approach was performed and a gross total resection was achieved.
Results: Microscopic findings of the tumor showed abundant anuclear laminated keratinaceous debris with a small focus of benign mature squamous epithelium. No adjacent adnexal structures were identified. Based on histopathological examination, the diagnosis was an epidermoid cyst. The patient's symptoms have resolved since the tumor resection and he will be followed with serial imaging for signs of tumor recurrence.
Conclusions: In summary, giant epidermoid cysts presenting outside the CP angle are rare lesions, but primary treatment remains surgical resection with a goal of achieving a complete removal of tumor to prevent recurrence.

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Extramedullary Spinal Presentation of Intracranial Mesenchymal Tumor with FET-CREB Fusion: Case Report with Literature Review

B Zeches[1], R Patton[2], J Jo[2], M Majeed[2], C Sippey[3], P Boyer[2], K Lee[3]
[1]Department of Pathology and Laboratory Medicine, East Carolina University; [2]East Carolina University; [3]ECU Health Neurosurgery

Background: Intracranial mesenchymal tumors (IMTs) are a recently recognized entity representing a group of neoplasms with FET-CREB fusions, typically presenting in children and young adults, with a spectrum of clinical behavior described, from slow growth to quick recurrence and metastasis. A majority of these neoplasms occur within the cranium with close dura association. Herein, we report a case of a spinal IMT. Case Report: A 53-year-old woman with right thoracolumbar region pain, several-years duration, presented with new pain radiating to the right inguinal area with associated leg paresthesias. Spine magnetic resonance imaging revealed a contrast-enhancing right extramedullary T11 mass, 1.8 x 1.3 x 1.9 cm, with T11-T12 foramen extension. The patient underwent laminectomy with microsurgical resection.
Methods: The patient's electronic medical records and all imaging studies were thoroughly reviewed. A PubMed.gov and Google.com literature search was conducted using appropriate key words.
Results: Histologic evaluation of the lesion revealed a predominantly spindle cell neoplasm with ovoid to rounded nuclei. The cells are separated by moderate amounts of extracellular matrix material, containing glycosaminoglycans with admixed collagen banding. Immunoprofiling revealed focal expression of epithelial membrane antigen and S-100 with diffuse expression of desmin, E-cadherin, CD99, and somatostatin receptor 2 (SSTR2). Fluorescence in situ hybridization revealed an Ewing sarcoma (EWSR1) gene rearrangement. Chromosomal microarray and fusion / transcript analysis identified a EWSR1:CREB1 fusion. Combined findings were consistent with diagnosis as IMT with FET:CREB fusion. Postoperative and 6-month imaging identified stable nodular enhancement, 0.8 x 0.8 cm, adjacent to the T11 pedicle. Two other spinal IMTs have been reported in the literature.
Conclusions: The differential diagnosis of an extramedullary spinal column lesion in an adult includes schwannoma, meningioma, solitary fibrous tumor, and ependymoma. While rare, the differential should also include IMT. Biopsy or resection

with histologic, immunohistochemical, and molecular characterization is necessary for definitive diagnosis.

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Pineal Melanocytic Neoplasm with Diffuse Leptomeningeal Dissemination: A Rare Primary Melanocytic Disease Process

B Zeches[1], K Dickerson[2], J Jo[2], H Kanaan[3], P Boyer[2]
[1]Department of Pathology and Laboratory Medicine, East Carolina University; [2]East Carolina University; [3]ECU Health Neurosurgery

Background: Metastatic melanoma commonly metastasizes to the brain and represents the most common melanocytic disease process affecting the central nervous system (CNS). However, as melanocytes are present within the arachnoid, primary melanocytic lesions also occur including melanocytomas, intermediate grade melanocytic lesions, and malignant melanomas, with classification based on the absence or presence of brain invasion along with mitotic activity and Ki67 proliferation index. We present a case of a patient with a primary melanocytic disease process. Case Report: A 50-year-old woman presented with confusion and speech alterations. Magnetic resonance imaging identified a 2.0 cm T1 hyperintense pineal mass with extension along the thalami, tectal plate, and superior cerebellum and involvement of the cerebral hemisphere leptomeninges. The differential diagnosis included a melanocytic neoplasm, pineal parenchymal tumor (e.g. pineoblastoma), germ cell tumor, and metastatic disease.
Methods: The patient's electronic medical records and imaging studies were thoroughly reviewed. A PubMed.gov literature search was conducted using appropriate key words.
Results: Biopsy of a right temporal lobe lesion identified neoplastic cells, some containing pigment, involving subarachnoid space and neuroparenchyma with Virchow-Robin space invasion, consistent with an intermediate grade melanocytic lesion and compatible with leptomeningeal dissemination from a primary pineal lesion. Immunohistochemistry for BRAF V600E was negative and molecular evaluation revealed an alteration in the BAP1 gene but no alteration in BRAF, KIT, or NRAS. These findings argue against metastatic melanoma which typically manifests multiple genetic alterations. As imaging has demonstrated aggressive biologic activity from a presumed pineal primary, the patient is undergoing chemotherapy to treat disseminated disease. Retinal examination was negative for uveal lesions.
Conclusions: While the vast majority of melanocytic neoplasms affecting the CNS represent metastatic melanomas, primary melanocytic neoplasms can rarely be identified. Clinicians, radiologists, and pathologists must be aware of this disease entity to avoid confusion with a metastatic disease process.

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Intraventricular Lesions in Adults: Meningioma and Solitary Fibrous Tumor Are in the Differential Diagnosis

B Zeches[1], C Sippey[2], M Majeed[3], J Jo[3], K Lee[2], P Boyer[3]
[1]Department of Pathology and Laboratory Medicine, East Carolina University; [2]ECU Health Neurosurgery; [3]East Carolina University

Background: Intraventricular meningiomas are uncommon but well-described lesions. Solitary fibrous tumors (SFTs) are rare neoplasms of the dura arising from mesenchymal cells and only rarely arising within the ventricles. Herein, we describe two patients with intraventricular lesions indistinguishable by neuroimaging, a fibrous meningioma and a SFT. Patient A: Imaging of the head of a 26-year-old woman with a history of seizures identified multiple extraaxial, dura-based lesions and a right lateral ventricle lesion, 4.4 x 3.6 x 4.0 cm. Resection of the lesion revealed a spindle-shaped neoplasm with interposed collagen and epithelial membrane antigen and E-cadherin expression, consistent with a fibrous meningioma, World Health Organization (WHO) grade 2 based on focal brain invasion. No recurrence has been detected on imaging one year after resection. Patient B: A 39-year-old woman presented with worsening headaches. Imaging identified a left lateral ventricle lesion, 5.2 x 4.9 x 4.0 cm. Resection revealed a neoplasm composed of ovoid nuclei and occasional staghorn blood vessels. Neoplastic cells showed STAT6, BCL2, and CD99 immunoreactivity. The diagnosis of SFT, WHO grade 3 was made using 2016 criteria, revised to grade 2 using 2021 criteria. Two years of surveillance imaging has identified no evidence of recurrence.
Methods: The patient's electronic medical records and imaging studies were reviewed. A PubMed.gov literature search was conducted using appropriate key words.
Results: Meningiomas constitute up to 14% of intraventricular neoplasms. Solitary fibrous tumors are rare intracranial neoplasms that constitute less than one percent of central nervous system tumors. A literature review revealed 29 cases of intraventricular SFTs with 91% located in the lateral ventricles, similar to the predominantly lateral ventricle occurrence of intraventricular meningiomas.
Conclusions: The differential diagnosis of an intraventricular lesion in an adult typically includes meningioma. While rare, the differential should also include SFT. Histologic and

immunohistochemical evaluation is necessary to make the appropriate diagnosis.

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Histiocytic Sarcoma of the CNS

R Cecchi, S Ferris
University of Michigan

Background: 59-year-old male with multiple enhancing brain lesions (involving both hemispheres). The patient underwent biopsy of a right frontal lobe lesion.
Methods: N/A
Results: H&E sections show a mesenchymal-appearing neoplasm, composed of cells with elongate sarcomatoid nuclei and moderately abundant eosinophilic cytoplasm. Some areas demonstrate spindled and fascicular architecture. Rare grooved nuclei are present, and scattered ring or "horseshoe-shaped" nuclei are present. Scattered mitotic figures are present. Perivascular lymphocytic cuffing and scattered parenchymal lymphocytes are present. Focal microvascular proliferation is present. Extensive areas of necrosis are present. Adjacent brain parenchyma is present, and the tumor appears relatively well-circumscribed. Initial immunohistochemical and molecular evaluation (Chromosomal microarray identified 5q chromothripsis and CDKN2A/B homozygous deletion, and Tempus xT 648 gene panel testing demonstrated MTAP copy number loss) were non-specific, and a preliminary diagnosis of "sarcomatoid neoplasm" was rendered. Genome-wide DNA methylation profiling showed no match to DKFZ brain classifiers (v11b4 or v12.5), but demonstrated fairly high alignment with Langerhans Cell Histiocytosis (0.72) by DKFZ Sarcoma classifier. Subsequent immunohistochemistry demonstrated the tumor staining for CD163, CD68, PU.1, CD14, CD4, S100 (rare), and fascin, while CD1a and Langerin were negative. Based on this staining profile, a final integrated diagnosis of histiocytic sarcoma was rendered.
Conclusions: Since its original characterization in 1970, Histiocytic sarcoma (HS) has remained a rare tumor with only approximately 30 cases of primary CNS HS reported. The WHO Classification of Haematolymphoid Tumors supplies a diagnostic criterion as variably pleomorphic cells, positive immunostaining for two or more histiocytic markers (here CD163, PU.1, CD14, and others), and negativity for CD1a, langerin (CD207), CD21, and CD35. HS has an aggressive course with a reported median survival of 7 months and average survival of 24 months. We encourage further sharing of this challenging diagnostic entity to gain better awareness and understanding of its diagnosis and facilitate development of treatment methods.

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Molecular Characterization of Three Cases of Papillary Tumor of the Pineal Region

B Liechty, J Solomon, D Pisapia
Weill Cornell Medicine

Background: Papillary tumor of the pineal region (PTPR) is a relatively rare entity and the range of molecular findings found in this tumor remains to be elucidated. Here we present three cases of patients (ages 10, 11, and 49) with PTPR that underwent next generation sequencing.
Methods: All three tumors were interrogated with either the MSKCC IMPACT panel or the TSO500 NGS gene panels. 450K methylation array profiling was performed on the third case and assesed using the Brain Tumor Methylation Classifier Version 11 developed by the DKFZ.
Results: In both pediatric cases, PTEN alterations were detected (a frameshift deletion in case 1 and a D92Y missense mutation in case 2), in accordance with what has been described in previous series of PTPR, and in both of these cases there was concurrent evidence of chromosome 10 loss. Additional copy number alterations included gain of chromosome 8 (case 1) and loss of chromosome 22 (case 2). In a third case presenting in an adult, while methylation array profiling confirmed a profile match with PTPR subtype B (calibrated score 0.998), no PTEN alteration was detected and no relative chromosomal gains or losses were detected. Instead, this case demonstrated two distinct frameshift mutations in the CIC gene that are considered likely to represent compound heterozygous loss-of-function alterations. Additionally detected variants of unknown significance involved CREBBP and PTPRD (case 1), and APC, ATM, and EIF1AX (case 3).
Conclusions: While CIC alterations have been described in other neoplasms arising in the CNS including oligodendroglioma and CIC-rearranged sarcoma, to our knowledge this is the first reported case of PTPR with CIC alterations. In this study we present the clinical, histopathological, and molecular findings in detail of three cases of PTPR.

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Melanocytic colonization in meningioma: Case report with a review of the three reported cases

M Elnagdy, G Kleinman, S Ahuja
Westchester Medical Center

Background: Meningioma is the most common primary brain tumor. According to the WHO classification, histological types of meningioma include low-grade type, grade 1, and atypical type, grade 2, and anaplastic type, grade 3. Melanocytic colonization has been reported in multiple extra-cranial tumors while only three cases of meningioma reported. Melanin pigment normally exists in the leptomeningeal dendritic cells. In this study, we are reporting the fourth meningioma case showing melanocytic colonization and doing a comparative review showing interesting findings compared to the other three reported cases to date.
Methods: Hematoxylin and Eosin staining for representative tumor sections was done according to our laboratory protocol. Immunohistochemistry was done using: GFAP, EMA, CD163, Ki-67, PR, S100, HMB45, and MART1. Statistical analysis was performed using Microsoft excel and GraphPad prism.

Results: Tumor showed meningothelial cells arranged in nest and whorls. High mitotic activity (~5 mitotic figures/10 HPFs and ~20-30% Ki-67 positivity). Moreover, Brain invasion highlighted by GFAP staining was identified. These findings were consistent with atypical meningioma. On immunohistochemistry, Tumor cells were positive for EMA, CD163 and negative for PR. Focal areas showed pigmented cells which were immunopositive for melanocytic markers e.g., MelanA. Our case shares a lot of similarities with the other reported cases. Gender, race and the histological grade (atypical meningioma with brain invasion) were similar.
Conclusions: We are reporting a case of atypical meningioma, WHO grade 2 with melanocytic colonization. We did a comparison between the findings in our case and other cases. Based on comparison, it appears to be more associated with brain invasive atypical meningioma, WHO grade 2. The role of gender and race in the pathology of this finding is yet to be clarified. Incidence of this pathological phenomenon in WHO grade 1 meningiomas and the mechanisms involved in the colonization process need more research.

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Diffuse Large B-Cell Lymphoma of the Brain and Intravascular Large B-cell Lymphoma in the Same Patient: Which Came First?

R Sobel, A Saleem, B Chul Yoon, F Fei, H Lau
Palo Alto VAHCS and Stanford University School of Medicine

Background: The relationships between intravascular large B-cell lymphoma (IVLBCL) and diffuse large B-cell lymphoma (DLBCL) arising in the CNS and other immune-privileged sites are unclear.
Methods: We report the case of a 90-year-old man with mild dementia who presented with subacute altered mental status; neuroimaging demonstrated non-communicating hydrocephalus with cerebral aqueductal narrowing but without obvious mass lesion. Because of his age and multiple comorbidities, he was not a candidate for intervention and was placed in hospice care where he expired from aspiration pneumonia.
Results: Autopsy demonstrated IVLBCL in pericardial vessels and vessels within the tunica vasculosa of the testis, but not involving any other organs, including skin. Neuropathology demonstrated Alzheimer disease and DLBCL with subependymal spread, aqueduct obstruction, focal infiltration of dura and pituitary, but no intravascular component identified. The brain and non-CNS intravascular lymphomas were CD45+, CD20+, BCL6+, MUM1+, and CD10-, and HHV8- by immunohistochemistry, and EBER- by in situ hybridization. We hypothesized that despite morphologic and immunophenotypic similarities, molecular genetic factors might account for differences in organ tropism and intra- vs. extravascular localization. Targeted next-generation sequencing performed on brain and testicular samples detected 4 pathogenic/likely pathogenic mutations and 10 variants of uncertain significance (VUS) that were identical between the brain and intravascular lymphomas. The IVLBCL had 1 additional pathogenic variant, TET2 p.R1465, and 1 additional VUS, PTPN11 p.H520fs.
Conclusions: IVLBCL commonly involves the CNS but it is considered to be, "characterized exclusively by intravascular growth" (WHO Classification of CNS Tumours, 2021). Although the brain lymphoma is most likely a primary DLBCL of the CNS with the IVLBCL representing secondary systemic spread, ongoing RNA sequencing may further elucidate transcriptomic contributions to the distinct trafficking and other differences between the tumors that are relevant to pathogenetic mechanisms in IVLBCL and primary CNS DLBCL. (Supported by Stanford Gift Funds).

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Meningeal solitary fibrous tumor: A genome-wide DNA methylation study

K Eschbacher[1], Q Tran[2], S Jenkins[3], K Fritchie[4], E Moskalev[5], R Stoehr[5], A Caron[3], M Link[3], P Brown[3], A Guajardo[6], D Brat[7], A Wu[8], S Santagata[9], D Louis[10], P Brastianos[10], A Kaplan[10], B Alexander[10], S Rossi[11], F Ferrarese[12], D Raleigh[8], M Nguyen[8], J Gross[6], J Velázquez Vega[13], F Rodriguez[14], A Perry[8], M Martinez-Lage Alvarez[10], B Orr[2], F Haller[5], C Giannini[3]
[1]University of Iowa Hospitals and Clinics; [2]St. Jude Children's Research Hospital; [3]Mayo Clinic; [4]Cleveland Clinic; [5]Institute of Pathology, University Hospital Erlangen; [6]John Hopkins University; [7]Northwestern University Feinberg School of Medicine; [8]University of California San Francisco; [9]Brigham and Women's Hospital; [10]Massachusetts General Hospital; [11]Treviso General Hospital; [12]Ospedale Ca'Foncello; [13]Emory University Hospital; [14]University of California Los Angeles

Background: In a cohort of 126 patients (57 F, 69 M; mean age 53.0 years) with pathologically confirmed meningeal solitary fibrous tumors (SFT), we previously found that risk of metastasis was significantly associated with 2021 CNS WHO grade ($p = 0.005$), whereas NAB2::STAT6 fusion status ($n = 101$ cases; 51 = ex5-7::ex16-17, 26 = ex4::ex2-3; 12 = ex2-3::exANY/other and 12 =no fusion) was associated with disease specific survival ($p = 0.014$). TERT promoter mutation was identified in 11 (of 99; 11.2%) patients.
Methods: Genome-wide DNA methylation screening, successfully performed in 80 cases, revealed three distinct clusters: cluster 1 ($n = 38$), cluster 2 ($n = 22$), and cluster 3 ($n = 20$).
Results: Methylation clusters were significantly associated with fusion type ($p < 0.001$), with cluster 2 harboring ex4::ex2-3 fusion in 16 (of 20; 80.0 %) , nearly all TERT promoter mutations (7 of 8; 87.5 %), and predominantly an "SFT" histologic phenotype (15 of 22; 68.2 %). Clusters 1 and 3 were less distinct, both dominated by tumors having ex5-7::ex16-17 fusion (respectively 25 of 33; 75.8%, and 12 of 18; 66.7%) and with variable histological phenotypes, respectively "SFT" ($n = 7$, 18.4%; $n = 9$; 45.0%), "HPC-like" ($n = 12$, 31.6%; $n = 4$, 20.0%), or intermediate ($n = 19$, 50.0%; $n = 7$, 35.0%).

Methylation clusters were significantly associated with metastasis-free survival (10-year estimates: 67.0%, 94.7%, 25.0% for clusters 1, 2, and 3, respectively; $p = 0.0134$), but not overall survival, recurrence-free survival, or progression-free survival.

Conclusions: In summary, methylation clusters were significantly associated with fusion type, TERT promoter mutation status, histologic phenotype, and metastasis-free survival. Further analysis of association of specific methylation clusters with patient outcomes is ongoing.

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Whole Genome Cell-Free Tumor DNA Liquid Biopsy for Ultrasensitive Detection of Low Grade Meningiomas

L Geiser Roberts[1], L Geiser Roberts[1], I Tran[1], D Afterman[2], S Phillips[2], S Deochand[2], I Tavassoly[2], E White[2], R Kandasamy[2], P Polak[2], B Oklander[2], C Sen[1], D Pacione[1], J Golfinos[1], A Zviran[2], M Snuderl[1]
[1]NYU Langone Medical Center; [2]C2i Genomics

Background: Detecting tumor mutations in plasma cell-free tumor DNA (ctDNA) enables minimally invasive monitoring. However, it is significantly more sensitive in high grade tumors with high mutational burden. We examined whether ctDNA whole genome sequencing (WGS) utilizing noncoding mutation signatures can be used for monitoring in low grade meningiomas.

Methods: We evaluated 9 CNS WHO grade 1 meningiomas. WGS was performed on matched tumor and normal DNA from blood with 40x coverage, and ctDNA, extracted from 1-2mL plasma, with 20x coverage. We derived a personalized mutational pattern for each tumor and used an AI-based error suppression model for quantification and ultra-sensitive detection of ctDNA. A personalized mutational signature for ctDNA detection from WGS was quantified and the tumor fraction (TF) was compared to imaging, mutational burden and methylation profiling. Mutational drivers were analyzed using NYU Langone Genome PACT DNA NGS assay and whole genome DNA methylation and meningioma classifier.

Results: Four meningiomas were skull base and 5 hemispheric ranging in size 2.1-6.9 cm. Exonic somatic mutations ranged from 0-3 with a median of 1. 5/9 had NF2 mutations and/or LOH. Methylation subclasses were benign or intermediate. 6/9 had a detectable plasma ctDNA with TF ranging from 4.34E-6 - 1.65E-4. Detectable TF levels showed no correlation with size, location, mutations or methylation class. The copy number variation (CNV) profiles were flat in 2 cases, showed loss of Ch22q in 5 cases, aneuploidy in 1 case and loss of Ch1p, 8 and 22q in 1 case. All cases with 22q loss were ctDNA positive, whereas the cases with flat CNV profiles and the case with aneuploidy were negative.

Conclusions: We show that WGS using the non-coding DNA mutational signatures can detect ctDNA in low grade meningiomas in plasma despite low exonic mutational burden. ctDNA WGS could enable post-operative monitoring of meningiomas.

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Multiple Tumors in a 30-year-old Man with SMARCB1/INI1 Germline Mosaicism

L Kozanno[1], E Brown[2], Y Fu[2], J Jordan[1], A Stemmer-Rachamimov[1]
[1]Massachusetts General Hospital; [2]University of Alabama Birmingham

Background: SMARCB1/INI1 loss is a driver of multiple tumors. In the CNS, germline mutations resulting in total loss of SMARCB1/INI1 expression are associated with the development of atypical teratoid/rhabdoid tumors (AT/RT), while hypomorphic germline mutations leading to partial loss of SMARCB1/INI1 are associated with familial schwannomatosis, a syndrome characterized by multiple schwannomas and meningiomas. Recent studies have also defined a subset of CNS tumors showing complete loss of SMARCB1/INI1 but lacking characteristic rhabdoid features, the cribriform neuroepithelial tumor (CRINET) – composed of epithelioid cells arranged in a cribriform pattern. These histological features raise the differential diagnosis with metastatic carcinoma and choroid plexus carcinoma. Although morphologically different, CRINETs and AT/RT have similar genetic profiles and reported CRINET cases show similar epigenetic profiles to AT/RT, subclass TYR.

Methods: A 29-year-old man presented with right-sided hearing loss, tinnitus, diplopia, confusion, and dizziness. Radiologic studies showed a large fourth ventricular mass and multiple masses in cranial nerve roots and in the cauda equina, favored to represent multiple schwannomas.

Results: Resection of the fourth ventricular mass revealed a malignant epithelioid neoplasm, which raised the differential diagnosis of metastatic adenocarcinoma. Immunohistochemical workup showed loss of SMARCB1/INI1, and the overall immunoprofile was consistent with cribriform neuroepithelial tumor (CRINET). Germline genetic testing revealed a mosaic out of frame deletion of exon 7 of SMARCB1/INI1. The patient passed away 4.5 years later. At autopsy, all spinal and cranial nerve lesions were found to be epithelial masses, which by immunohistochemistry and methylation analyses were shown to be metastatic deposits of the CRINET. No schwannomas were identified.

Conclusions: This is a report of an unusual case of a CRINET arising in a patient with a mosaic SMARCB1/INI1 pathogenic variant. In contrast to most CRINETs which present in the pediatric population and have favorable outcomes, our patient presented as an adult and had an aggressive course.

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The Spectrum of Central Nervous System Metastasis in Pediatric Population: A Meta-Analysis

N Leelatian, J Liang
Vanderbilt University Medical Center

Background: While central nervous system (CNS) metastasis is a minor portion of CNS tumors in children, they are associ-

ated with clinical deterioration and dissemination. Mechanisms of CNS metastases include distant spread and direct extension; however, previous studies often included cases with the former mechanism only. Since locations of CNS involvement may differ between tumor types, understanding the mechanisms of spread and locations of CNS involvement could guide clinical monitoring and management.

Methods: We performed a PubMed search using terms related to CNS metastasis in pediatric population (21 years of age or younger, in accordance with the American Academy of Pediatrics). We recorded the type of primary neoplasms (solid and hematolymphoid neoplasms), site of origin, age at diagnosis and CNS metastasis, gender, location of CNS involvement, and mode of CNS spread.

Results: We retrieved 4,284 articles. 556 articles (4,128 cases) met the criteria and were included. The most common types of neoplasm were leukemia (1,333, 32.3%), neuroblastoma (791, 19.2%), retinoblastoma (563, 13.6%), and rhabdomyosarcoma (360, 8.7%). The most common systems of origin were hematolymphoid (1,355, 32.8%), eye (564, 13.7%), and head and neck (300, 7.3%). Mesoblastic nephroma, malignant rhabdoid tumor of the kidney, and extracranial extrarenal rhabdoid tumor were entities that occur and metastasize to the CNS within the first year of life. Distant metastasis was the mechanism of CNS involvement in 81% of cases while the remaining 19% showed direct extension. Direct CNS extension most occurred in neoplasms originating in the eye and head and neck. Solid neoplasms commonly involved the brain while leptomeninges/cerebrospinal fluid and cranial nerve involvement most occurred in hematolymphoid neoplasms and retinoblastoma, respectively.

Conclusions: The mechanisms of spread and location of CNS involvement were distinct between different types of primary neoplasms. This study highlights the importance of understanding the mechanisms and sites of CNS spread for disease monitoring and management.

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Primary Central Nervous System Classic Hodgkin Lymphoma: A Rare Presentation

U Naik, S Ak, X Zhang, J Armstrong
The University of Texas Health Science Center at Houston

Background: Primary central nervous (CNS) classic Hodgkin lymphoma (CHL) is rare, with about twenty cases reported in the literature. We report a case of primary CNS CHL, emphasizing on the differential diagnosis and immunohistochemical workup.

Methods: An 83-year-old Hispanic female presented with progressive weakness, frequent falls, nausea and vomiting. Brain MRI showed a solid enhancing extra-axial mass in the right posterior cranial fossa (1.7 cm), which increased in size over 8 weeks (2.5 cm), causing hydrocephalus. No other lesions were identified elsewhere. Emergent sub-occipital craniectomy for resection of tentorium-based tumor and its cerebellar extension was performed.

Results: Histopathologic evaluation of the mass showed scattered atypical large lymphoid cells admixed within a mixed inflammatory infiltrate, consisting of small lymphocytes, plasma cells, numerous eosinophils, increased histiocytes and occasional neutrophils. The atypical large lymphoid cells showed one to multiple pleomorphic nuclei, vesicular chromatin, prominent nucleoli and abundant cytoplasm, some showing cytoplasmic retraction artifact. Occasional hypereosinophilic and pyknotic cells, patchy sclerosis and foci of necrosis were noted. Immunohistochemically, the atypical large cells are positive for CD30, CD15, MUM-1 (strong), PAX5 (weak), OCT2 (partial, weak), BOB1 (partial, weak) and EBER in situ hybridization, and negative for CD45, CD20, CD79a and T-cell markers.

Conclusions: The polymorphous inflammatory background with HRS-like cells raised a differential diagnosis of CHL, EBV-positive diffuse large B cell lymphoma (EBV+ DLBCL) and angioimmunoblastic T cell lymphoma (AITL). EBV+ DLBCL was not favored due to weak PAX5 expression and positivity for CD15, and the background T-cell population showed no immunohistochemical aberrancies, excluding AITL. HRS-like cells can also be seen in T-cell/histiocyte-rich large B cell lymphoma (THRLBCL), anaplastic large cell lymphoma (ALCL), lymphomatoid granulomatosis (LyG), and polymorphous lymphoproliferative disease. When challenged by specific histomorphologic features, it is critical to perform strategic immunohistochemical evaluation for accurate diagnosis of rare entities like primary CNS CHL.

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High-Risk Neuroblastoma with Recurrence 28 Years after Initial Diagnosis: A Case Report

M Kritselis[1], J Boxerman[1], B DeNardo[1], H Mistry[1], A Perez-Atayde[2], M Punsoni[1]
[1]Rhode Island Hospital; [2]Boston Children's Hospital

Background: Neuroblastoma (NB) is the most common neoplasm during the first year of life, and the most common extracranial solid tumor in the first two years of life. Patients with NB are classified into risk-groups based on a combination of predictive factors, with variable patterns of event-free survival. In particular, high-risk neuroblastoma (HR-NB), which accounts for approximately half of all patient's diagnosed with NB, was originally associated with a $< 50\%$ 5-year event-free survival. While these odds have improved over time due to advances in treatment, 50-60% of children with HR-NB experience relapse or fail to respond to neoadjuvant therapy, with a median progression-free survival of 6.4 months, overall 4-year progression-free survival of 6%, and survival rates of 20%.

Methods: While the median time from diagnosis to first relapse/progression ranges from 18.7 to 22 months, there are rare cases of late recurrence. Very rarely patients have been reported to develop late recurrence of NB up to 20 years after the initial diagnoses.

Results: Herein we present the first reported case of an individual with high-risk neuroblastoma initially diagnosed at two

years of age, who presented with spinal cord compression secondary to an extradural neuroblastoma 28 years following initial diagnosis.
Conclusions: This novel report reinforces that close monitoring is important in patients with HR-NB, as well as suggests that extreme outliers exist, and additional investigation of these individuals may contribute to our understanding of this common and deadly disease.

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Spatial molecular imaging of immune microenvironment in brain metastases

I Caliskan, J Phillips
University of California San Francisco

Background: The brain is a common site of metastatic breast cancer and data from murine models and human tumor samples suggest both tumor cell-intrinsic and tumor cell-extrinsic factors contribute to brain metastasis. Indeed, there is a growing appreciation for the role of tumor-stroma interactions in supporting the metastatic niche. Yet, the specific factors involved in tumor-stroma crosstalk in the brain versus in other metastatic sites remains unclear.
Methods: A decedent with invasive ductal carcinoma of the breast widely metastatic to the brainstem, thalamus, temporal cortex, liver, skeletal muscle, bone, bilateral ovaries, and lungs was chosen to identify the metastasis-associated factors and compare them across organs in a single patient. To investigate both spatial- and organ-specific signaling networks at the different metastatic sites, we utilized multiplex immunostaining and spatial transcriptomics with single-cell resolution using CosMX Spatial Molecular Imager (NanoString Technologies, Seattle, WA, USA). Tissue microarrays were generated from regions of interest, including dense tumor and tumor interface regions at each organ. Each region was then subjected to spatially defined multiplexed RNA and protein profiling.
Results: A total of 60,522 cells and 14.1 million transcripts were profiled. Using the protein data and deconvoluted RNA expression data, we are phenotyping the innate and adaptive immune cells and characterizing ligand-receptor pairs involved in tumor-stroma crosstalk within and across metastatic sites. In the interface regions, the density of CD3+ T cells and IBA1+ monocytes was highest in the lung and lowest in the brain ($p = 0.01$ and $p = 0.007$ with pairwise comparison, respectively). Within each metastatic site, IBA1+ monocyte density was greatest in tumor versus interface while CD3+ T cells density was greatest in the interface versus tumor.
Conclusions: By comparing the protein and RNA expression data across metastatic sites within the same patient, our goal is to identify organ-specific signaling networks that modulate the immune response and promote metastatic outgrowth.

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What is the best way to use Ki67 in the neuropathologic workup of meningiomas?

M McCord, V Thirunavu, M Drumm, A Steffens, M Youngblood, H Smith, J Walshon, K McCortney, S Magill, C Horbinski
Northwestern University

Background: Ki67 immunohistochemistry has long provided additional prognostic information in meningiomas. Prior studies establishing its role mostly used manual counting, which is laborious and subject to interobserver variability. It also remains unclear whether the output should be average Ki67 over an entire slide versus a "hot spot," and whether it matters which tissue block is immunostained in a given case.
Methods: To answer these questions, we used a retrospective cohort of 121 newly diagnosed meningiomas (65 Grade 1, 37 Grade 2, and 19 Grade 3), each of which had a minimum of 5 years clinical follow-up. Each case was annotated with patient age and sex, extent of resection, and WHO grade. All slides within each meningioma (average 7 blocks/tumor) were immunostained for Ki67, digitally scanned, and analyzed via QuPath to objectively quantify overall average Ki67 per slide, as well as the 2.2 mm2 area (10 high-power fields) within each slide that had the highest Ki67 percentage (i.e., the "hot spot"). One block per case was designated as the "best block" based on size and subjective impression of its overall representativeness. Variables showing significant association with recurrence-free survival (RFS) and overall survival (OS) on univariate analyses were then tested in multivariate Cox proportional hazards models.
Results: Based on multivariate Cox proportional hazards models, variables independently associated with shorter RFS included WHO Grade 3 (HR:4.79, 95% CI:1.20-17.90, $p = 0.026$), WHO Grade 2 (HR:2.43, 95% CI:1.01-5.83, $p = 0.047$), subtotal resection (HR:7.72, 95% CI:3.25-18.37, $p < 0.001$), and maximum hot spot Ki67 among all blocks (HR:1.07, 95% CI:1.01-1.13, $p = 0.024$). In contrast, shorter OS variables included male sex (HR:13.98, 95% CI:3.15-62.05, $p < 0.001$) and average Ki67 over the entire best block (HR:1.48, 95% CI:1.04-2.10, $p = 0.030$).
Conclusions: These data suggest that digital quantification of average Ki67 over one entire selected best block may ultimately provide the most useful information for overall patient survival.

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Intratumoral abscess: Case report of a rare cause of non-spontaneous necrosis in a meningioma

P Nisarga, J Ahn, F Chan, K Gyure
Allegheny Health Network

Background: Hematogenous spread is the dominant route of infection transmission to the central nervous system (CNS). There has been no direct association between CNS infections and tumors, however anecdotal reports in literature have reported both pathologies in the same patient.
Methods: We present an 85 year old deceased woman with multiple co-morbid conditions, prior intractable Citrobacter koseri urinary tract infection and an incidental falcine meningioma with intratumoral abscess.
Results: The patient had no prior history of seizures or dementia. She presented with altered mental status, anomic aphasia and fixation on certain subjects, along with focal seizures. Meningitis and encephalitis panels were negative, as were urine and blood cultures. Magnetic resonance imaging showed bilateral calvarial enhancement and a left parafalcine contrast-enhancing mass. Autopsy neuropathologic examination revealed a 1.3 cm left parafalcine (parietal) meningioma; microscopic examination showed a grade 1 meningioma with a focal intratumoral abscess characterized by acute inflammation and necrosis. No other high-grade features were present. Tissue Gram stain and PAS histochemistry were negative for bacterial and fungal organisms, respectively.
Conclusions: Meningioma with abscess (intratumoral and peritumoral) has been infrequently described and seen in association with a clinical history of infection, including urinary tract infection. Patients have presented with symptoms including drowsiness, fever, headache, personality change, focal neurologic deficits, hemiparesis, focal seizures, and status epilepticus. Hematogenous spread is thought to be the most likely pathogenesis of abscess formation in meningioma. Although necrosis alone does not affect the grade of meningioma, its presence may upgrade the tumor given other atypical features. The main cause of non-spontaneous necrosis in meningioma is preoperative embolization, but rarely, intratumoral/peritumoral abscess may need to be ruled out given the appropriate clinical setting.

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Metastatic Melanoma of an Unknown Primary Site: A Case Report

A Vila, S Sakthi-Velavan
Marian University College of Osteopathic Medicine

Background: Metastatic melanoma (MM) is refractory to treatment, and patients with MM have a median survival of 6 months. A donor in the anatomy laboratory was reported to have MM as the cause of death. However, the findings were unique, suggestive of a rare presentation or a co-existing pathological entity. The case report explores the unique combination of cutaneous and visceral lesions that may be inadvertently interpreted to be of non-malignant etiology.
Methods: The study investigated the pathology noted in a 75-year-old female donor, with MM as the reported cause of death, during routine dissection in the anatomy laboratory. Detailed dissection, examination, biopsy, and histopathological examination (HPE) of the lesions were done. Photographs of the lesions on the skin and viscera were taken.
Results: Multiple slightly elevated lesions of 2-3 cm diameter were found on the gluteal region and anterior abdominal wall. Several firm and smooth nodules of around 1 cm diameter were found on the pericardium, pleura, peritoneum, diaphragm, lungs, heart, stomach, intestines, liver, spleen, kidneys, uterus, retroperitoneal region, and mediastinum. HPE of skin lesions showed basaloid proliferation and dermal hyperplasia. The visceral lesions showed multifocal tumors with small, loosely arranged eosinophilic cells and dense triangular nuclei. Immunohistochemical staining (pan-cytokeratin AE1/AE3, Melan A, and Ki-67) were performed, and the results are awaited.
Conclusions: The most common sites of metastasis of cutaneous melanoma are lymph nodes, liver, lungs, and brain; however, lesions were not found in the brain of this donor. Extensive visceral involvement and generalized distribution of nodules of MM may be inadvertently interpreted to be of non-neoplastic origin. In immunosuppressed patients, the multisystemic visceral lesions may be confused for granulomatous diseases such as sarcoidosis, brucellosis, histoplasmosis, or miliary tuberculosis. Since melanoma is immunogenic and more common in immunosuppressed patients, the clinical presentation warrants further evaluation.

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A rare presentation of a pediatric intracranial mesenchymal tumor with FET::CREB fusion

R Teixeira-Yokoda, M Vij, M Umphlett, J Crary, N Tsankova, J Walker, T Richardson
Icahn School of Medicine at Mount Sinai

Background: An 18-year-old male with a history of Stage IV Wilms tumor, status-post nephrectomy, chemotherapy, and radiation at 8 years of age was evaluated for headaches in the right orbital region. MRI demonstrated a 2.4 x 1.4 cm extra-axial, heterogeneously-enhancing, solid and cystic mass on the right sphenoid wing, suggestive of either a meningioma or Wilms tumor metastasis.
Methods: Case report
Results: Intra-operative pathology consult showed a highly cellular neoplasm with epithelioid and rhabdoid features. Permanent sections showed a well-circumscribed hypercellular neoplasm, arranged in lobules, with focal whorling and occasional nuclear clearing, admixed with prominent rhabdoid morphology. There were multiple myxoid foci, a capsule with a lymphocytic rim, and areas of increased lymphoid aggregates forming occasional germinal centers. Immunohistochemistry was positive for EMA, PR in a subset of cells, CD99, and Des-

min. INI-1 and BAP-1 were retained. WT1 and STAT6 were negative. Ki-67 proliferation index was 4.5%. Next generation sequencing (NGS) revealed a EWSR1::ATF1 fusion gene. The diagnosis of intracranial mesenchymal non-meningiothelial tumor with a FET::CREB fusion was established, specifically Angiomatoid Fibrous Histiocytoma (AFH) with EWSR1::ATF1 fusion. This is the first sphenoid wing pediatric tumor in this category diagnosed at Mount Sinai Hospital. The patient is being closely monitored, given the uncertain behavior or these tumors. There is epigenetic data to support a different methylation signature from the non-intracranial soft tissue tumors.

Conclusions: The diagnosis of AFH is made by a combination of histomorphology, immunophenotype, and a proven EWSR1::ATF1 fusion. These tumors often mimic meningioma, and in the current case, additional NGS was performed due to the patient history and the prominent lymphocytic rim. Without the NGS result, the diagnosis could not have been established.

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Atypical teratoid/rhabdoid tumor of the sella in an adult patient

B Morales Vargas[1], T Garzon[1], J Velázquez Vega[2], M Abedalthagafi[1], M Schniederjan[2], S Neill[1]
[1]Emory University School of Medicine; [2]Children's Healthcare of Atlanta/Emory University

Background: Atypical teratoid/rhabdoid tumor (AT/RT) is a malignant embryonal neoplasm that is classically described primarily in pediatric patients. Recently, a subset of these tumors has been noted occurring within or nearby the sella turcica in adult patients and particularly in adult females. Genomic studies have detected some slightly different aberrations in these "sellar AT/RTs" compared to the tumors typically found in pediatric patients; many of the sellar AT/RTs grouped with the MYC subtype of AT/RTs by methylation profiling. Histologically, sellar AT/RTs can show a range of appearances overlapping with other morphologically heterogenous neoplasms found in the sella.

Methods: Immunohistochemical staining showed focal expression of synaptophysin, EMA and CAM5.2 as well as loss of SMARCB1 expression. Next-generation sequencing detected dual inactivating SMARCB1 mutations. Methylation profiling confirmed a MYC subtype AT/RT.

Results: We report the case of a 60-year-old woman who presented with a right third cranial nerve palsy. MRI disclosed a 1.3 cm mass in the sella with involvement of the right cavernous sinus. Surgical resection was pursued. Intraoperative smears and frozen section displayed a cellular neoplasm comprised of dyshesive, relatively monotonous cells exhibiting a high nuclear to cytoplasmic ratio. Histologic examination of permanent sections revealed similar highly atypical cells in addition to foci of necrosis and readily identifiable mitotic figures. No characteristic rhabdoid cells were identified.

Conclusions: Sellar AT/RTs comprise a rare but clinicopathologically unique group of tumors that are important to recognize, given their aggressive behavior and the necessity of multimodal treatment. As in this case, some of the morphologic features can overlap with those of other more common and less aggressive neoplasms. Thus, sellar AT/RTs are a key, albeit rare, part of the differential diagnosis of sellar neoplasia.

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Cavernous Sinus Sclerosing Epithelioid Fibrosarcoma: Unique Presentation of a Rare Entity

Y De Los Santos[1], N Wick[1], J Gagan[2], J Bishop[2], K Hatanpaa[2]
[1]UT Southwestern Medical Center, Dallas;Parkland Health and Hospital System; [2]UT Southwestern Medical Center, Dallas

Background: Sclerosing epithelioid fibrosarcoma (SEF) is a rare tumor most often arising in the upper and lower extremities or limb girdle, followed by the trunk and head and neck regions. Only four previous cases of SEF arising in the neural axis have been reported.

Methods: A thirty-seven-year-old female presented with left eye pain and ophthalmoparesis. Magnetic resonance imaging (MRI) revealed a 1.8 x 1.7 cm enhancing mass in the left cavernous sinus. The pituitary gland was unremarkable. The top radiology differential included meningioma versus schwannoma. The patient underwent a transsphenoidal endoscopic biopsy of the mass. A postoperative MRI obtained four weeks after the biopsy for radiation planning purposes demonstrated progression of the tumor into the left orbit. The patient is currently undergoing radiotherapy.

Results: The intraoperative smear demonstrated a unique finely granular background with a monolayer of epithelioid cells, mild-moderate nuclear pleomorphism, and finely granular cytoplasm. The frozen section contained predominantly cords of epithelioid cells embedded in hyalinized stroma. There were no mitoses and the MIB-1 proliferation index was low, approximately 2%. The tumor cells were diffusely and strongly positive for MUC4, beta-catenin, and vimentin. The cells were negative for CAM 5.2, SSTR2, EMA, STAT6, S100, synaptophysin, GFAP, TTF1, LCA, CD68, CD1a, CD34, SMA, and ALK. Next generation sequencing demonstrated an EWSR1-CREB3L2 fusion.

Conclusions: The essential diagnostic criteria of SEF include epithelioid, MUC4-positive and keratin-negative cells in trabeculae, nests, or cords embedded in a hyalinized stroma. A characteristic fusion is helpful but not required for the diagnosis. The most frequent fusion reported in $>60\%$ of cases is EWSR1-CREB3L1. However, CREB3L2/L3 and CREM are also reported as fusion partners. Awareness of this entity is

crucial for neuropathologists. The radiologic features mimicking benign tumors such as meningioma and schwannoma, along with the deceptively bland low-grade histology, represent a potential diagnostic pitfall.

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Sarcoma arising in the setting of treatment naive neuroendocrine neoplasia

B Maveal[1], V Duncan[2], M Lora Gonzalez[2]
[1]University of Alabama Birmingham; [2]University of Alabama

Background: We report two cases of treatment naïve sarcomas arising within neuroendocrine neoplasia. The first case is a 56-year-old female with a spindle cell lesion found to be primary sella rhabdomyosarcoma appearing in the background of an atypical pituitary adenoma/pituitary neuroendocrine tumor (PitNET). The second is a 40-year-old male with a sphenoid nasopharyngeal tumor with a biphasic morphology consisting of epithelioid and spindle cell/sarcoma morphology.
Methods: Immunohistochemistry
Results: The Pit-NET spindle cell component was inexorably progressive with subsequent resections and radiation treatment. It was retrospectively found to be desmin and myogenin positive as reported (Primary Sellar Rhabdomyosarcoma Arising in Association with a Pituitary Adenoma, Duncan et al.). The second case showed the sarcoma component is positive for synaptophysin, chromogranin, S-100, GFAP, desmin, myogenin, and myoD1 while negative for keratins, ACTH, SOX10, and SF-1. The epithelial component is positive for synaptophysin, chromogranin, ACTH, and SSTR2, with focal SF-1 and PIT-1, with the epithelioid component being negative for desmin, myogenin, myoD1, SOX10, and S100.
Conclusions: These two unique cases illustrate the rarity of sarcomas arising in the setting of treatment naïve patients in conjunction with neuroendocrine neoplasms/features, the need for further studies in such entities, and a heightened awareness of stromal/spindle cell lesions within endocrine neoplasms

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Low-Grade Ghost Cell Odontogenic Carcinoma of the Sella

G Sexton, D Marker
University of Pittsburgh Medical Center

Background: Ghost cell odontogenic carcinoma (GCOC) is a rare odontogenic neoplasm that most commonly arises in the maxilla and mandible, either de novo or from benign precursor lesions showing overlapping histology but lacking malignant features. GCOC has also been reported in the sellar region as a form of malignant transformation of craniopharyngioma (CP), and has been shown to harbor beta-catenin mutations in some cases.
Methods: We present a case of a low-grade GCOC arising in the sellar region of a young female patient who initially presented with symptoms of endocrine dysfunction. Workup initially revealed a solid and cystic mass in the sellar region, which was biopsied and diagnosed as CP. The patient received radiation therapy. Three years later, imaging showed an increase in size of the lesion and she was scheduled for resection.
Results: The resection specimen showed a tumor composed of sheets of basaloid epithelial cells with loose, stellate reticulum-like foci, scattered ghost cells, abundant dentinoid-like matrix, and invasion of surrounding brain parenchyma. Apoptotic bodies and mitotic activity were present to varying degrees throughout. On immunohistochemistry, scattered aggregates of cells showed nuclear beta-catenin staining, including the cells in the matrix, and an overlapping population showed nuclear staining for LEF-1. Next-generation sequencing confirmed a beta-catenin mutation (p.T41I) and no matching class was found on methylation profiling (Heidelberg classifiers v11b4 and v12.5).
Conclusions: While the tumor in our case showed parenchymal invasion, proliferative activity, and cytologic atypia not in keeping with the histologically similar benign precursors of GCOC, it lacked the necrosis and markedly increased proliferative activity commonly seen in GCOC and was thus designated as low-grade. GCOC and adamantinomatous CP show significant overlap in histologic features, raising interesting questions regarding the origin of both tumors and highlighting the importance of their accurate recognition by neuropathologists.

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An Unusual Case of Metastasis to Meningioma

C Perez, H Kellermier
Summa Health System - Akron City Hospital

Background: Tumor-to-tumor metastasis (TTM) is a rare metastatic phenomenon in which a primary malignant tumor metastasizes to another tumor. The most common intracranial recipients of systemic metastases are meningiomas, which constitute approximately one fifth of all intracranial tumors. The most common origins of TTM are breast and lung carcinomas. Due to their rarity, the clinical characteristics of these patients are elusive.
Methods: N/A
Results: We report the case of a 67-year-old male patient with known history of a WHO grade 2 atypical meningioma and prostatic adenocarcinoma metastatic to bone. He presented due to increased seizure activity and, upon imaging, an extra-axial enhancing mass was identified. This mass was located in the high right frontal region, at the site of a previously resected meningioma. The mass was believed to be the recurrent meningioma and subsequently, a craniotomy was performed. Intraoperative consultation of the mass was compatible with the patient's history of atypical meningioma with definitive diagnosis deferred to permanents. Upon histological examination, the mass was found to be a metastatic prostatic carcinoma within a WHO grade 3 anaplastic meningioma.

Conclusions: There have been 12 cases of prostatic adenocarcinoma with TTM to meningioma reported so far in the literature. The pre-operative diagnosis of TTM remains a challenge. Multiple epidemiological and pathophysiological mechanisms have been proposed including the increase in incidence of metastases and meningiomas with age and the hypervascularity of meningiomas, which increases the chances of receiving hematogenous metastasis. Even though clinical and neuroimaging features may be critical in the suspicion of TTM, there are no factors specific for this phenomenon.

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Low-grade neuroepithelial tumors with EWSR1::PLAGL1 fusion

Y Suh, S Kim, S Kim, S Park
Seoul National University Hospital

Background: Pleomorphic adenoma gene-like 1 (PLAGL1) fusion-positive CNS tumors are extremely rare. This gene fusions have been reported in the morphological diagnosis of ependymomas and unique neuroepithelial tumors that showed a biphasic pattern of neuroepithelial differentiation.
Methods: Here, we report the clinicopathological findings and the biological behavior of two low-grade neuroepithelial tumors (NET), EWSR1::PLAGL1-fused with immunohistochemical and NGS studies.
Results: They occurred in the left frontal cortex of a 3-year-old girl and the left temporoparietal angular gyrus of a 16-year-old girl. Both patients had presented with seizures. Histopathologically, they exhibited identical biphasic patterns; sheets of mixed small round neurocytic and ganglion cells with abundant neuropil background. This biphasic pattern mimicked subependymoma because of its alternative cellular area and fibrillary matrix at a low-power field. Both tumors were robustly positive for GFAP and synaptophysin and were studded NeuN-positive small neurocyte-like and ganglion cells. NF stain revealed rich axons (neuropils) between the tumor cells. In addition, small tumor cells were focally positive for Olig2 and Sox10 and ganglion cells were positive for phosphorylated mTOR. However, entire tumor cells are negative for BRAF VE1, IDH1, nestin, CD34, CD99, and Lin28A. There were no high-grade features with scarce mitoses and low Ki-67 index. RNA sequencing resulted in EWSR1::PLAGL1 fusion and a methylation study classified these two tumors into a neuroepithelial tumor with PLAGL1-fused in both cases (NET_PLAGL1_FUS, by v12.5 DKFZ methylation class algorithm). There was no evidence of recurrence in both patient during the 33-month and 12-month follow-up periods.
Conclusions: This study may reveal more knowledge about neuroepithelial tumor, EWSR1::PLAGL1 fused.

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Dedifferentiated Meningeal Solitary Fibrous Tumor

J Persons, K Eschbacher
University of Iowa Hospitals and Clinics

Background: Solitary fibrous tumors (SFTs) are characterized by the presence of a NAB2::STAT6 fusion. These tumors may rarely undergo dedifferentiation, wherein the more conventional-appearing areas become admixed with foci of extreme pleomorphism, resembling sarcoma. Within the soft tissue, dedifferentiated SFT has been associated with TERT promotor and TP53 mutations. Although the body of literature on meningeal dedifferentiated SFT is sparse, a small number of cases demonstrating TP53 mutation have been reported.
Methods: In this report, we describe a case of meningeal dedifferentiated SFT.
Results: The patient was a 43-year-old woman who initially presented with a left occipital mass that was surgically resected and determined to be SFT. She underwent post-operative radiation and was later lost to follow-up. She returned twenty years following her initial resection with several months' history of progressive visual decline. An MRI brain demonstrated an extra-axial, homogenously enhancing occipital mass encasing the superior sagittal sinus. The mass was subsequently resected and histologically was comprised of a densely cellular tumor with haphazardly arranged cells and intervening thin-walled, dilated, branching vessels. Mitotic activity and focal necrosis were identified. Several areas demonstrated marked nuclear atypia with an undifferentiated pleomorphic sarcoma-like appearance. Both components demonstrated STAT6 nuclear positivity. More widespread TP53 overexpression was present in the dedifferentiated areas. RNA fusion testing further demonstrated a NAB2::STAT6 fusion in both components (NAB2-exon 5::STAT6-exon 17). Next generation sequencing revealed TERT promotor and TP53 mutations within the dedifferentiated component only. Given these histologic, immunohistochemical, and molecular features, the tumor was determined to be a dedifferentiated SFT, CNS WHO Grade 3.
Conclusions: Meningeal SFTs have the propensity for local recurrence and metastasis, even late in the disease course; however, cases of dedifferentiation are rare. An important management challenge is that it can be difficult to predict eventual tumor dedifferentiation based on initial presentation. Long-term surveillance of all patients is recommended.

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Atypical meningioma with YAP1-MAML2 fusion: A case report and review of the literature

A Nobee, Y Rong, A Seth, N Jhala, I Akhtar
Temple University Hospital

Background: Less than 1% of meningiomas occur in the pediatric and adolescent/young adult (AYA) population without sex bias. YAP1 fusion has been found to be an oncogenic driver for sporadic pediatric and AYA meningiomas. Here, we report a case of atypical meningioma with brain invasion and YAP1-MAML2 fusion.
Methods: A 20-year-old male with no past medical history presented with a seizure for the first time and was found to have a 1.4 x 1.1 cm enhancing lesion in the right medial temporal lobe within the cortex and white matter. The lesion was resected.

H&E sections demonstrated meningothelial cells associated with calcifications, collagen-rich stroma, and a marked lymphohistiocytic infiltrate. The neoplastic cells were positive for SSTR2a, PR, and E-cadherin, but negative for EMA and D2-40. GFAP demonstrated focal brain invasion. Molecular studies revealed YAP1-MAML2 fusion. Work-up for hematolymphoid neoplasm was negative.
Results: NF2 is an upstream negative regulator of YAP signaling and loss of the NF2 protein product, Merlin, results in YAP overexpression and target gene transcription. PYGO1, FAM118B, and MAML2 are reported partners for YAP1. YAP1-MAML2 fusion is mainly found in low-grade pediatric meningiomas with rhabdoid features and can be NF2-independent.
Conclusions: We identified a YAP1-MAML2 atypical meningioma with brain invasion located within the brain parenchyma. Larger cohort studies are needed to determine a possible prognostic role of YAP1 alterations in meningiomas.

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CNS solitary fibrous tumor with unique papillary histologic features

Z Xiong
Department of Pathology and Laboratory Medicine, Saint Like's Health system/UMKC School of Medicine

Background: CNS solitary fibrous tumor is a relatively uncommon primary CNS tumor. It is usually dura-based and with characteristic histologic morphology of haphazardly or in short, ill-defined fascicles or sheets of monomorphous spindle cells and intratumoral staghorn vessels. It characteristically harbors NAB2-STAT6 gene fusion. Here, we report a case of CNS solitary fibrous tumor that exhibited uniform papillary histologic features.
Methods: A seventy-one years old male presented to hospital with a remote history of prostate cancer and complained with right lower extremity weakness. Brain MRI examination found a 6 cm extra-axial heterogeneously enhancing mass arising from the falx in the left parietal region. Surgical resection of the tumor was performed afterwards. The resulted specimen was submitted for pathologic evaluation.
Results: The specimen was received as fragmented pink-gray, rubbery to friable tissue. The tissue was further processed for hematoxylin and eosin stained sections. Microscopically, the tumor was composed of diffuse micro-papillary structures with fibro-vascular core covered by one to two layers of atypical cuboidal cells with hyperchromatic and pleomorphic nuclei and high N/C ratio. There were 6-7 mitoses per 10 high power fields. Definitive necrosis was not seen. Immunohistochemical evaluation showed that the neoplastic cells were positive for CD99 and negative for epithelial membrane antigen, glial fibrillary acidic protein, synaptophysin, chromogranin, prostate-specific antigen, and prostatic acid phosphatase. Molecular study demonstrated a NAB2::STAT6 gene fusion.
Conclusions: Based on the clinical information, histopathology and molecular alteration, this tumor was classified as solitary fibrous tumor, CNS WHO grade 2. This case further confirms that there is a papillary variant of solitary fibrous tumor, in addition to the conventional morphology.

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Merlin expression in human meningiomas – An immunohistochemical study

S Torp, R Meta, S Tollefsen, I Vestrheim, W Sjursen
Norwegian University of Science and Technology (NTNU) / St. Olavs University Hospital

Background: Merlin is a tumor suppressor protein encoded by the NF2 gene (Neurofibromatosis type 2). Mutations in this gene and merlin inactivation are commonly encountered in spontaneous meningiomas and schwannomas. Studies on expression and clinical significance in human meningiomas have shown somewhat diverging results. The aim of this study was to investigate the expression of merlin by means of immunohistochemistry and relate these findings to clinicopathological parameters in a series of human meningiomas.
Methods: Merlin immunohistochemistry was performed on 171 human meningiomas including two NF2 cases. Twenty cases also underwent NF2 gene analyses.
Results: All cases were immunoreactive. In 12 out of 20 cases NF2 gene mutations were detected. Merlin expression was found not to be associated with histological subtype, localization, tumor grade, or time to recurrence.
Conclusions: As all meningiomas were immunoreactive for merlin, immunohistochemistry is not the method of choice to reveal NF2 gene aberrations. The merlin expression did not reveal any clinical significance.

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Atypical teratoid rhabdoid tumor-TYR subtype arising in the setting of germline ring chromosome 22: An uncommon form of tumor predisposition

J Lee[1], Q Tran[2], R McGee[2], M Perrino[2], S Upadhyaya[3], N Pytel[2], A Carroll[4], W Orisme[2], M Eldomery[2], L Wang[2], P Balckburn[2], L Furtado[2], A Viaene[5], M Luo[5], J Kalish[5], B Orr[2]
[1]St. Jude Children's Research Hospital; [2]St. Jude Children's Research Hospital, Memphis, TN; [3]C. S. Mott Children's Hospital, University of Michigan, Ann Arbor, MI; [4]University of Alabama at Birmingham, AL; [5]Children's Hospital of Philadelphia, PA

Background: Atypical teratoid/rhabdoid tumor (ATRT) is an embryonal neoplasm that often includes rhabdoid cells and polyphenotypic differentiation. Biallelic inactivation of SMARCB1, or less commonly SMARCA4, is the defining molecular alteration of ATRT. While rhabdoid tumor predisposition syndrome with germline inactivation of SMARCB1 or SMARCA4 is widely recognized and evaluated for in patients with ATRT, a less common form of ATRT predisposition is the presence of germline ring chromosome 22. Germline ring chromosome 22, though

not disruptive of SMARCB1 directly, is inherently unstable and prone to loss creating predisposition to somatic mosaicism for monosomy chromosome 22. Furthermore, deletion or disruption of distal genes such as SHANK3 during ring formation of chromosome 22 can result in Phelan McDermid syndrome (OMIM #606232) or other phenotypic sequelae.

Methods: Herein is presented one such example, to raise awareness of this alternative mechanism for germline predisposition to ATRT and potential associated clinical manifestations, with detailed tumor and germline molecular characterization.

Results: The patient presented as a 23-month-old male with developmental delay, right foot macrodactyly, and dysmorphic facial features. He was found to have a posterior fossa ATRT, germline ring chromosome 22 [r(22)(p11.2q13.31] with a terminal 4.87 Mb deletion of 22q involving SHANK3 and other genes but not SMARCB1, and monosomy chromosome 22 in the tumor sample with focal homozygous deletion of SMARCB1. Additionally, there was an unbalanced germline translocation of uncertain significance der(18)t(X/Y;18)(p22.33/p11.32;p11.32). Methylation profiling of the tumor demonstrated a calibrated score of 0.93 for ATRT, subclass TYR. To the best of our knowledge, this is the first example of ATRT arising in the setting of germline ring chromosome 22 with reporting of methylation profiling and ATRT subtype results.

Conclusions: In ATRT patients with somatic SMARCB1 inactivation and wildtype germline SMARCB1, germline karyotype and chromosomal microarray could be considered, especially for patients with dysmorphic features, developmental delay, or disproportionate growth.

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Digital Analysis of Spatial Heterogeneity in Ki-67 Staining of Meningioma

B Faust[1], M DiStasio[2], H Tang[2], U Ugwuowo[2]
[1]Department of Pathology, Yale School of Medicine; [2]Yale School of Medicine

Background: Ki-67 staining by immunohistochemistry is an important adjunct to H&E mitotic index in evaluating the proliferative activity of meningioma. Standard strategies for evaluation of both of these quantities rely on pathologist selection of the region likely to contain the highest fraction of proliferating cells. Regional heterogeneity within meningioma tissue can complicate the appropriate selection of these regions, particularly in subtly varying tumors. It may also represent an unquantified source of variation in overall clinical behavior.

Methods: In order to more precisely characterize regional variation in the proliferative index of meningiomas, we retrieved from the archives of the Yale Department of Pathology 136 sequential cases of meningioma (including grades 1, 2, and 3) on which Ki-67 immunostaining was performed, digitized them by whole slide imaging, and employed a publicly available IHC segmentation algorithm in QuPath software to comprehensively identify positive and negative nuclei. We randomly selected a subset of regions on every slide for manual validation, and also compared computed proliferation indices to those included in the associated clinical reports.

Results: 31 cases passed strict manual quality control across the whole image, and among those, there was good agreement between pathologists' overall assessment of tumor proliferative activity and digital analysis (Pearson's product-moment correlation 0.81). However, the complete distribution of proliferative fractions in regions throughout each tumor revealed substantial heterogeneity (coefficient of variation 0.47), not captured by a single summary value as is typically reported.

Conclusions: These findings have implications for the parameters used for implementing routine use of computer-assisted Ki-67 quantification in clinical practice, and for the study of regional variability in proliferation capacity in meningioma. Future studies investigating the relationship between regional distributions of proliferation fraction and clinical behavior in these tumors are needed to address the predictive utility of quantifying this variability.

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TROP2 Immunohistochemical Expression in Meningiomas

H Ozkizilkaya[1], H Ozkizilkaya[2], O Basit Ogunbona[2], F DeMonte[2], C Kamiya Matsuoka[2], C Lopez-Garcia[3], Y Esquenazi[4], L Ballester[2]
[1]The University of Texas MD Anderson Cancer Center Translational Molecular Pathology Department; [2]The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [3]Hospital San José TecSalud, Tecnológico de Monterrey, Monterrey,Nuevo León, Mexico; [4]The University of Texas Health Science Center at Houston, Vivian L. Smith Department of Neurosurgery, Houston, TX, USA. Center for Precision Health, School of Biomedical Informatics, The University of Texas Health Science Center at Houston, Houston, TX, USA. Memorial Hermann Hospital-TMC, Houston, TX, USA

Background: Meningiomas represent the most common primary brain tumor in adults. While most meningiomas can be effectively treated with surgery, complete resection is not always feasible in all anatomic locations and some meningiomas recur despite successful surgeries. The availability of drugs with known efficacy for the treatment of meningiomas is limited. TROP2 (Human trophoblastic cell surface antigen) has been identified as a potential target for cancer therapy in breast, bladder, head and neck and lung carcinomas with various ongoing clinical trials. In this study, we evaluate the expression of TROP2 in meningiomas to assess its potential as a therapeutic target.

Methods: We identified 50 meningiomas from our institutional database diagnosed between 2016-2019 (26-grade 1, 16-grade 2, and 8-grade 3). Clinicopathologic characteristics were collected from EMR. Tissue Microarrays (TMA) were stained with TROP2 antibody. Median mitotic count and Ki-67 index were assessed by experienced neuropathologists. Progression Free Survival (PFS) and Overall Survival (OS) were analyzed using a Log-rank test.

Results: Median age at diagnosis was 53.5 years. Eighteen (36%) patients were male, and 32 (64%) were female. Average mitotic count (H&E) for grades 1, 2, and 3 were 1, 5, and 25, respectively. The average Ki67 labelling index correlated with WHO grade and was quantified as 2.5 % (grade 1), 8.9 % (grade 2) and 35.8% (grade 3). TROP2 was positive in 5 cases, 2 grade 1 (7.7 %), 1 grade 2 (6.2 %), and 2 grade 3 (25%). No differences in survival based on TROP2 expression were identified.
Conclusions: Our findings suggest that TROP2 is expressed in a subset of cells in ∼10% of meningiomas. The role of TROP2 inhibitors in the treatment of meningioma patients remains to be studied.

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A Rare Unusual Plurihormonal Pituitary Neuroendocrine Tumor with Multi-lineage Differentiation Presenting in a Patient with Acromegaly

C Jaramillo, C Biehl, N Clement
Brooke Army Medical Center

Background: Pituitary neuroendocrine tumors (PitNETs, pituitary adenomas) are clonal neoplasms of adenohypophyseal origin classically located in the sellar region. PitNETs are classified according to cell of origin into TPIT-lineage corticotroph tumors, SF1 gonadotroph tumors, PIT1-lineage tumors, and PitNETs with no distinct cell lineage.
Methods: Here, we relate the case of a 37-year-old male who presented with a right medial subconjunctival hemorrhage and intermittent headaches. A non-contrast CT Head demonstrated a 1.3 cm sellar mass. History was remarkable for increasing hat, shoe, and ring sizes with concomitant coarsening of facial features. Labs showed an elevated IGF-1 (918 ng/mL). MRI Brain with and without contrast redemonstrated a 1.3 cm well-circumscribed T2-hypointense non-enhancing sellar mass consistent with a pituitary macroadenoma. A transsphenoidal resection was performed.
Results: Histologic sections demonstrated a diffuse tumor composed of monomorphic cells with bland nuclei. Immunohistochemistry for transcription factors demonstrated that the tumor cells were strongly positive for both SF1 and PIT-1 and negative for T-PIT. Staining for hormonal markers showed that many tumor cells were strongly positive for LH and weakly positive for GH, some were positive for prolactin, and very rare cells were positive for TSH and ACTH. Staining for for FSH was negative. Cam 5.2 demonstrated diffuse granular staining in most cells and rare fibrous-like bodies in $< 10\%$ of cells. Ki-67 index was 2.6%.
Conclusions: These findings are diagnostic of PitNET with no distinct cell lineage, subtype plurihormonal tumor. Descriptions of this rare tumor are limited to case reports; its pathogenesis is not well understood, though it has been posited they represent a stem cell tumor with multilineage differentiation. The purpose of this report is to raise awareness of this subtype and encourage a comprehensive immunohistochemical workup for accurate classification of PitNETs.

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Iatrogenic immunodeficiency-associated lymphoproliferative disorder -different terminology in hematopathology and neuropathology

R Teixeira-Yokoda, M Vij, M Umphlett, J Crary, N Tsankova, J Walker, A Duffield, T Richardson
Icahn School of Medicine at Mount Sinai

Background: The WHO classification of central nervous system (CNS) tumors (5th edition) includes hematolymphoid subclassification of immunodeficiency-associated (IDA) CNS lymphomas. The etiology of the immunodeficiency includes primary immune disorders, autoimmune disorders, HIV, underlying neoplasms, and iatrogenic immunosuppression. These IDA CNS lymphomas differ from primary diffuse large B-cell lymphoma (DLBCL) of the CNS in that they, by definition, arise in an immunodeficient patient and are EBV-positive. In systemic lymphomas, the distinction between DLBCL, NOS and IDA DLBCL is clinically important because the tumors associated with iatrogenic immunosuppression may show partial regression in response to immunosuppressive drug withdrawal. Here we highlight two cases of CNS lymphoma associated with iatrogenic immunosuppression.
Methods: Case report
Results: Both cases are first-time diagnoses of large B-cell lymphomas confined to the CNS. The first case is a 69-year-old woman with a history of pauci-immune rapidly progressive glomerulonephritis treated with chronic mycophenolate mofetil. In this case, the immunophenotype was characteristic for DLBCL (positive for CD20, PAX5, MUM1, CD30, BCL-2, P53, and EBV), and meets the criteria for a formal diagnosis of IDA CNS lymphoma. The second case is a 57-year-old woman with a history of rheumatoid arthritis who was treated with chronic methotrexate. This case also shows an immunophenotype characteristic for diffuse large B-cell lymphoma. However, in this case, the neoplastic cells are EBV-negative; thus, the neoplasm does not meet the formal criteria for classification as an IDA CNS lymphoma. In contrast, hematopathologists classify hematolymphoid neoplasms in immunosuppressed patients as IDA lymphoproliferative disorders regardless of EBV expression.
Conclusions: These two cases illustrate the possibility of developing CNS lymphoma in patients with iatrogenic immunosuppression, highlight the importance of clinical

correlation in the subclassification of hematolymphoid neoplasms, and suggest that expanding the definition IDA CNS lymphomas to include both EBV-positive and EBV-negative tumors may be warranted.

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Sellar Paraganglioma: The Existence Dilemma

R Hoyt[1], V Davoudi[2], N Lakis[1], M Haeri[1,2]
[1]Department of Pathology and Laboratory Medicine, University of Kansas Medical Center; [2]University of Kansas Alzheimer Disease Research Center

Background: Paragangliomas (PGL) are benign vascular tumors arising from neural crest progenitor cells. Head and neck PGLs are frequently located beside parasympathetic paraganglia of the vagus and glossopharyngeal nerves. Primary sellar PGLs are extremely rare, and to our knowledge only 39 cases have been reported so far. The origin of sellar PGL is proposed to be from intrapituitary embryonic remnants of paraganglion cells or migrated preganglionic cells found in tympanic/ciliary branches of the glossopharyngeal nerve. Paraganglioma in the sellar region was initially reported by Bilbao et al. (1978). Patients with sellar primary PGL are mostly adults and most commonly present with symptoms of non-functioning pituitary adenoma. No sign of catecholamine excess has been reported in these patients. Instead, most patients suffered from headaches, visual disturbances and ophthalmoplegia like symptoms seen in pituitary adenomas. Null cell adenomas, on the other hand, are tumors found in the sellar region with negative immunoreactivity for all pituitary hormones and transcription factors by definition and may histologically look similar to PGLs.
Methods: To examine the possibility of misdiagnosis of a null cell adenoma for PGL we reviewed the literature for reported sellar PGLs.
Results: The most relevant immunohistochemical (IHC) studies to differentiate the 2 entities were examined. Immunoreactivity to S100 was reported in 16 cases and the rest were either negative (7 cases) or were not performed. Interestingly, none of the cases reported IHC for pituitary transcription factors (Pit-1, TPIT, SF-1). Furthermore, we examined 2 cases of presumed sellar PGLs in our center, which were negative for all pituitary hormones and transcription factors, supporting the diagnosis of PGL. However, methylation profiling of both tumors matched gonadotroph pituitary adenoma with high confidence.
Conclusions: We conclude that sellar paraganglioma, if it truly exists, is extremely rare, and a full workup is essential to differentiate it from more common mimics.

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Elevated expression of caveolin-1 in primary diffuse large B-cell lymphomas of the CNS correlates with patient's poor survival

Y Sugita[1], K Takahashi[2], K Higaki[2], T Furuta[3], M Moritsubo[3], H Takahashi[4], A Kakita[4]
[1]St.Mary's Hospital; [2]St. Mary's Hospital; [3]Kurume University School of Medicine; [4]Brain Research Institute, Niigata University

Background: Caveolin-1 (Cav-1) is a component of the vesicle-like structure of the plasma membrane called caveolae. Cav-1 is involved in the transport of substances from the plasma membrane to the intracellular space of various cells, including CNS cells. Cav-1 has been reported to be involved in growth mechanisms, neoangiogenesis and immune evasion in various malignant tumors. In this study, we investigated the relationship between Cav-1 expression in primary diffuse large B-cell lymphomas of the CNS (CNS-DLBCLs) and clinicopathological characteristics in order to clarify the role of Cav-1 in the progression of CNS-DLBCLs.
Methods: Forty CNS-DLBCLs diagnosed at Departments of Pathology, Kurume University, St. Mary's Hospital, and Brain Research Institute, Niigata University were included. The expression of Cav-1 in tumor cells and proliferating vascular endothelial cells was examined by immunostaining. The patients were classified into three groups according to the labeling rate: low (L) ($0< L < 30\%$), intermediate (I) ($30\%< I < 60\%$), and high (H) ($60\%< H$). The relationship between each group and clinical parameters was statistically analyzed.
Results: The patients with CNS-DLBCLs consisted of 25 males and 15 females. Twenty patients were over 65 years old. Adjuvant therapies consisted of radiation therapy (R) in eight patients, chemotherapy (C) in 11 patients, and R+C in 20 patients. One patient did not receive any adjuvant therapies. All cases were Cav-1 positive. Thirteen, 16, and 11 patients were in groups L, I, and H, respectively. Statistical analysis showed that patients in the H group had lower survival rates than those in the L and I groups (Log-rank test, $p < 0.01$). On the other hand, other clinical factors (gender, age, radiation, chemotherapy) did not correlate with the prognosis of patients with CNS-DLBCLs.
Conclusions: Overexpression of Cav-1 in CNS-DLBCLs correlated with patient's poor survival and tumor progression. Immunostaining for Cav-1 is useful for grading CNS-DLBCLs.

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Methylation signature critical for diagnosis of unusual metastatic spine lesion originating from intraventricular choroid plexus papilloma

B Faust[1], M Aboian[2], J Baehring[2], A Huttner[2], J Moliterno[2], B Orr[3], A Darbinyan[2]
[1]Department of Pathology, Yale School of Medicine; [2]Yale University; [3]St. Jude Children's Research Hospital

Background: DNA methylation array profiling is an effective and relevant diagnostic tool in the approach to metastatic lesions of unknown origin. Particularly challenging are metastatic spine lesions with an ambiguous histomorphologic or immunohistochemical pattern and an unknown or unusual site of origin. We present the case of a 77-year-old man who presented with a lumbosacral spinal mass lesion. He complained

of erectile dysfunction, rectal pain with radiation, urinary and stool incontinence. Imaging revealed an enhancing intradural, extramedullary lesion with mass effect on cauda equina roots and obliteration of CSF space.
Methods: Surgery was performed and histology revealed an epithelial neoplasm with papillary architecture.
Results: Nuclear and cellular atypia was present only focally with a mildly elevated proliferative index (Ki-67) ranging from 5-8%. Despite an extensive panel, immunohistochemical stains were inconclusive, showing positivity only for pan-cytokeratin, CAM5.2 and focal weak positivity for CK7, S100 and PSA. This pattern initially raised concerns for a metastatic adenocarcinoma of likely prostatic origin, however, this interpretation lacked clinical support as the patient had no known diagnosis or clinical signs of prostate cancer and PSA levels were normal. A molecular approach was chosen to identify the site of origin. Although whole exome sequencing was unrevealing, DNA methylome analysis surprisingly classified the neoplasm as a choroid plexus tumor. Subsequently, it was discovered that this patient had undergone surgery for a benign choroid plexus papilloma 40 years prior.
Conclusions: This is a highly unusual case of a benign choroid plexus papilloma which recurred at the site of a likely drop metastasis to the lumbosacral spine 40 years after initial resection. DNA methylome analysis was critical in aiding a difficult diagnosis where other modalities were suboptimal. This case highlights the diagnostic utility and power of performing methylation analysis in the diagnosis of metastatic lesions of unknown origin.

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A Case of CNS-Presenting Histiocytic Sarcoma

N Haslett, D Guptil, J DeWitt
University of Vermont Medical Center

Background: Here we present the case of a 6-year-old male who presented to his primary care doctor with three weeks of morning headaches, nausea, vomiting, and generalized fatigue (patient was falling asleep in his classroom). Subsequent MRI of the brain demonstrated a 3.3 x 2.9 x 2.9 cm, well-circumscribed, enhancing mass in the right frontal lobe with local mass effect and confluent surrounding edema causing 4 mm or leftward midline shift. Two days after MRI findings, the patient underwent right frontal craniotomy for tumor resection. Follow-up MRI demonstrated gross total resection of the right frontal mass.
Methods: Neuropathologic review showed a hypercellular tumor with mixed inflammation consisting of histiocytes, neutrophils, and plasma cells. There were aggregates of histiocyte-appearing cells that showed variably indented-to-irregular nuclei and prominent nucleoli. Additionally, there were large, pleomorphic, discohesive atypical-appearing cells with slightly eosinophilic cytoplasm that had a histiocytic appearance and displayed brisk mitotic activity. Ki67 highlighted elevated proliferative activity. Adjacent the hypercellular areas, there were foci of necrosis. Immunohistochemical workup showed tumor cells to be negative for GFAP, AE1/3, CD21, CD1a, Sox-10, Mart-1, CD30, ALK 1, and NX2/EP336. INI-1 was retained. Tumor cells were strongly positive for CD68, CD163, CD4, and S100, consistent with histiocytic origin. The overall findings were consistent with a malignant histiocytic proliferation consistent with histiocytic sarcoma.
Results: Methylation sequencing was performed, with no match identified to the CNS classifier, suggestive of a rare tumor, not of CNS histogenic origin. Molecular diagnostics detected a POLQ variant. Next Generation sequencing Fusion panel detected a MIGA2-BRAF fusion.
Conclusions: Overall, the histologic, immunohistochemical, and molecular findings support a diagnosis of histiocytic sarcoma arising in the CNS. While histiocytic sarcoma presenting in the CNS is a rare diagnosis, it has rarely been reported both in adult and pediatric populations.

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CNS Lymphoma in the ICC and WHO Classification: An Institutional Review of 22 Cases

B Price, B Cho
University of North Carolina School of Medicine

Background: With the release of the International Consensus Classification (ICC) and the fifth edition of the World Health Organization classification (WHO), there are now two major systems for categorizing hematolymphoid neoplasms of the central nervous system (CNS). Despite significant overlap, differences between these systems may manifest in neuropathology practice. Here we review 22 CNS lymphoma cases from the University of North Carolina (UNC), focusing on updates according to the new classifications.
Methods: NA
Results: Of the 22 CNS lymphomas diagnosed at UNC from 2018 to 2022, 13 were primary and 9 represented CNS involvement by systemic disease. 8 primary cases were large B-cell lymphoma (LBCL), now classified within "primary LBCL of immune-privileged sites" by the WHO and as "primary diffuse LBCL of the CNS" by the ICC. 2 LBCLs showed a germinal center B (GCB) immunophenotype; 6 showed a non-GCB immunophenotype. 1 case was EBV+. All were negative for MYC/BCL2 double expression. FISH for MYC, BCL2, and/or BCL6 rearrangement was performed in 7 cases; no double-hit lymphomas were identified. 4 of the 22 CNS lymphomas occurred in immunodeficiency settings: 3 in HIV+ patients and 1 in a kidney transplant recipient. 2 HIV+ patients had EBV+ LBCL, now diagnosed as "diffuse LBCL, EBV+, HIV setting" per the WHO. 1 HIV+ patient had an EBV+ polymorphic post-transplant lymphoproliferative disorder (PTLD)-like appearance, now called "polymorphic lymphoproliferative disorder arising in HIV" by the WHO. The kidney transplant recipient had an EBV+ monomorphic PTLD, LBCL, which the WHO now calls "diffuse LBCL, EBV+, post-transplant setting".
Conclusions: CNS LBCL is incorporated within the diagnosis of "primary LBCLs of immune-privileged sites" by the WHO

but not the ICC. Whereas the ICC retains PTLD terminology, the WHO has replaced it with a broader category of immune deficiency and dysregulation (IDD) utilizing a three-part nomenclature (histologic entity, EBV status, IDD setting).

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CNS Tumor with EP300::BCOR Fusion in a Patient with Neuroblastoma

S Belakhlef, S Sharma
Augusta University Medical Center

Background: CNS tumor with BCOR internal tandem duplication was initially described in pediatric population with variable histologic features. Recent studies suggest a broader age range and spectrum of BCOR alterations. We present a case of a CNS Tumor with EP300::BCOR Fusion. A 17-year old male with a history of a right neck neuroblastoma at 2-years old, following chemoradiation and stem cell transplant, presented with aura and headaches. MRI demonstrated a 2.5 cm heterogeneously enhancing mass of the right mesial temporal lobe concerning for metastatic neuroblastoma.
Methods: A right temporal craniotomy and tumor resection was performed.
Results: Histopathology demonstrated a proliferation of small, round-to-oval cells with occasional pseudorosettes. Mitoses were readily identified. The neoplastic cells were immunopositive for CD99, WT-1, BCL-2, Olig2, and FUS and negative for GFAP, S100, Phox-2B, synaptophysin, chromogranin, CD34, NeuN, and BCOR. P53 showed wild-type pattern. ATRX and INI-1 demonstrated retained nuclear expression. Ki-67 was markedly elevated. Next generation sequencing demonstrated an EP300::BCOR Fusion. Following radiation and temizolamide, the tumor recurred at age 19. Histopathology showed an infiltrative tumor with variable cellularity, fibrillary-to-myxoid microcystic stroma, thin-walled capillaries, and small uniform rounded nuclei with rare mitoses. The tumor was immunopositive for BCOR and Olig2 and negative for GFAP and synaptophysin. Methylation class was consistent with CNS high-grade neuroepithelial tumor with BCOR alteration, and positive for EP300::BCOR Fusion transcript by RNA-seq analysis.
Conclusions: We bring forth this case to expand the knowledge of this rare entity, its unusual presentation, better differentiated histology in the recurrence (possible maturation) and the limitations of immunohistochemistry, and molecular analysis. Tauziède-Espariat A, Uro-Coste E, Sievers P, et al. CNS tumor with EP300:: BCOR fusion: discussing its prevalence in adult population. Acta Neuropathol Comm 2023;11(1):26. Torre M, Meredith DM, Dubuc A, et al. Recurrent EP300-BCOR fusions in pediatric gliomas with distinct clinicopathologic features. J Neuropathol Exp Neurol 2019;78(4):305-14.

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Expression of hydrogen sulfide (H2S) producing enzyme cystathionine γ-lyase (CSE) in metastatic brain tumors

M Lechpammer[1], Z Rabow[2], T Morningstar[2], A Serganov[3], E Nudler[3]
[1]Foundation Medicine, Inc.; [2]School of Medicine, University of California Davis, Sacramento, CA; [3]New York University School of Medicine, New York, NY

Background: H2S is a cysteine metabolite and a gaseous mediator that plays multiple roles in bioenergetics, proliferation, and angiogenesis of solid malignancies, including those frequently spreading into the brain. Nonetheless, little is known about the H2S involvement in the intracranial spread of solid tumors. Previously our group observed that CSE is the major H2S producing enzyme in glioma cells.
Methods: In this study, we have retrospectively reviewed expression of CSE in metastases from 122 patients (51% women, 49% men; mean age: 56.3) who underwent surgical resection or biopsy for newly diagnosed brain masses. Metastases originated from the lung, breast, kidney, ovarian, liver, colorectal, and papillary thyroid carcinomas, as well as from unknown primary tumors.
Results: Moderate to high CSE expression was detected in 85% (22/26) NSCLC-adenocarcinoma, 80% (8/10) NSCLC-squamous, 57% (4/7) undifferentiated and 43% (7/16) lung neuroendocrine tumors brain metastases. H scores ranged from 72.3 in the lung neuroendocrine brain metastases to 142.7 in metastases from NSCLC-adenocarcinomas ($p = 0.038$). Moderate to high CSE expression was detected in 100% (3/3) liver, 55% (6/11) breast, 54% (7/13) colorectal, 38% (5/13) kidney and 36% (4/11) unknown primary cancer metastases to brain. One metastasis from ovarian carcinoma (17%; 1/6) and none (0/4) of metastases from papillary thyroid carcinoma expressed CSE. H score in liver cancer brain metastases was significantly higher than in other brain metastases regardless of the primary tumor of origin ($p < 0.01$).
Conclusions: Distinct expression patterns of CSE were observed in brain metastases depending on the primary tumor of origin, with lung and liver cancer brain metastases having higher CSE expressions than metastases from other cancers. Our findings emphasize the importance of cysteine metabolites in cancer intracranial spread and aggressiveness. Further studies are warranted to evaluate cysteine metabolites as prognostic markers or therapeutic targets to benefit patients with metastatic brain tumors with similar metabolic signature.

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Application of Stimulated Raman Histology as a Surrogate to Oil Red O for Intraoperative Diagnosis of Hemangioblastoma

M Movahed-Ezazi, M Nasir, D Alber, A Faustin, J Golfinos, M Snuderl, D Orringer, D Zagzag
NYU Langone Health

Background: Hemangioblastomas are highly vascular tumors arising in the central nervous system, mostly occurring sporadically with a quarter of cases associated with von Hippel-Lindau (VHL) disease. Histologically, a key feature to help distinguish these tumors from other neoplasms is the presence of lipid containing vacuoles that are identified intraoperatively by conventional method of frozen section and Oil Red O staining. Stimulated Raman histology (SRH), an ex-vivo optical imaging method that enables microscopic examination of fresh tissue intraoperatively, has been on the forefront of neuropathology as an alternative tool to frozen section analysis. Conventional Oil red O method is labor and time intensive and consumes tissue. SRH allows for imaging of fresh tissue revealing lipid vacuoles, and furthermore enabling remote telepathology review for rapid intraoperative diagnosis.
Methods: We performed intraoperative consultation for two patients from NYU Langone Health, with lesions of the cervicomedullary junction and posterior fossa, using 3-mm sized fragments of fresh, unprocessed tissue for biopsy. Each biopsy underwent intraoperative imaging using our portable, NIO Imaging System, a dual-wavelength fibre laser imaging machine that utilizes stimulated Raman scattering microscopy (SRS) which is a process that employs the intrinsic vibrational properties of lipids, proteins and nucleic acids to generate a real-time virtual-H&E image.
Results: Review of SRH images by neuropathologists showed vascular cellular lesions comprised of tumor cells displaying emptied-out cytoplasmic spaces with lipid containing vacuoles, consistent with a diagnosis of hemangioblastoma. Permanent section H&E slides further confirmed the diagnosis.
Conclusions: Our study reveals the utility of SRH imaging for intraoperative diagnosis of hemangioblastomas with identification of lipid vacuoles, a classic morphologic feature, and thus can be a surrogate for conventional frozen section and Oil Red O stain. Furthermore, the ability to enable remote telepathology review continues to deliver improved patient care by providing neurosurgeons with a more timely diagnosis and ensuing appropriate surgical management.

SATURDAY POSTERS: Neurodegenerative: Alzheimer

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Isolation of single neuronal nuclei by phospho-tau accumulation in Alzheimer's disease postmortem brain

S Naik[1], S Kirkham[2], A Huang[2], X Qian[2], Z Zhou[2], D Oakley[3], M Frosch[3], B Hyman[3], C Walsh[2], M Miller[4]
[1]Brigham and Women's Hospital; [2]Boston Children's Hospital; [3]Massachusetts General Hospital; [4]Brigham and Women's Hospital

Background: Tau misfolding is a prominent pathologic feature of Alzheimer's disease (AD) and other neurodegenerative tauopathy diseases. Tau, which generally localizes to the cytoplasmic cellular compartment and binds microtubules, is hyperphosphorylated in AD and misfolds into aggregates known as neurofibrillary tangles. Neurofibrillary tangles only burden a subset of neurons in AD-involved brain regions, for which the cause and pathogenic impact is not well understood. Comparison of individual cells with differential tau pathology may provide insight into disease pathogenesis and reveal therapeutic targets.
Methods: Based on our observation of phosphorylated tau (P-tau) signal with neuronal nuclei isolated from human brain, we developed a method to separate nuclei with distinct tau states using fluorescence-activated nuclear sorting (FANS).
Results: We found that P-tau aggregates adhere to the nucleus in AD, detected by FANS and visualized by immunofluorescence microscopy (IF). On FANS, we observed disease-specific P-tau signal on nuclei, such that advanced AD (Braak stage V-VI) could be readily distinguished from non-AD samples. We observed disease-specific single-nucleus tau signal with multiple tau antibodies. Confocal IF showed P-tau adherent to the outside of purified AD neuronal nuclei, paralleling the signal observed on FANS. To assess the biological relevance of the P-tau that is detected during FANS, we mixed AD and non-AD control samples and demonstrated with whole exome sequencing that P-tau+ nuclei originated from AD, representing the specific tau accumulation status of that neuron.
Conclusions: This method allows for the interrogation of human neuronal nuclei on the basis of single-cell tau pathology, enabling a diverse array of rapid-preparation genomic studies, including single-nucleus genomics, transcriptomics, and epigenomics to dissect the molecular signatures of neurodegeneration.

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Alzheimer disease pathologic changes in temporal lobectomies for epilepsy

K Han, S Magaki, R Diaz, C Williams, S Dewar, J Chang, K Vossel, J Engel, H Vinters
University of California, Los Angeles

Background: The time course of deposition within the human brain of pathologic lesions deemed important in the onset and progression of Alzheimer disease (AD), the most common cause of dementia affecting over 6 million individuals in the US alone, is unknown. These include the accumulation of Aβ peptide in the brain parenchyma as amyloid plaques and in the blood vessels as cerebral amyloid angiopathy (CAA), as well as the neuronal accumulation of neurofibrillary tangles (NFTs) comprised of hyperphosphorylated tau. The density of these

lesions correlates, albeit imperfectly, with severity of cognitive impairment, but it is unclear (1) when the lesions first become apparent in the brain and (2) their precise relationship with the progression and severity of cognitive impairment.

Methods: Temporal lobe and/or hippocampus specimens from patients who underwent surgery for medically intractable epilepsy were examined for AD neuropathologic changes, including amyloid plaques and NFTs, as well as for cerebral amyloid angiopathy, α-synuclein and TDP-43 pathology using immunohistochemistry.

Results: The patients ranged in age from 28 to 69 years with seizure duration ranging from 3 to 48 years. Preliminary examination of specimens from 14 patients demonstrated 4 patients with significant amyloid plaque deposition in the temporal lobe, 2 of whom also showed a mild to moderate density of NFTs in the temporal lobe and hippocampus. One patient with mild CAA had no amyloid plaques, and one patient had abundant NFTs in the hippocampus in the absence of amyloid deposition.

Conclusions: Although intractable epilepsy is a neurological disease thought to affect a largely different patient cohort from those with dementia, a subset of patients who underwent temporal lobectomy and/or hippocampectomy demonstrated AD lesions. Examining the two loci, in which some of the earliest AD changes occur, from these patients can provide a unique window into early stages and temporal evolution of AD pathology.

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Examining vascular markers in Alzheimer's Disease

E Bearer[1], M Ranjbar[1], K Sanchex[1], C Martinez[2], Y Yang[1], C Keene[3], G Rosenberg[1], K SantaCruz[1]
[1]University of New Mexico Health Sciences Center; [2]University of New Mexico Health Sciences Center; [3]University of Washington School of Medicine

Background: Cerebral vascular brain injury is often comorbid with Alzheimer's disease (AD). White matter changes from vascular injury alone may present with dementia. We are embarking on a study to identify biomarkers for small vessel disease in vascular cognitive impairment and dementia (VCID) and apply these biomarkers to AD. Two matrix-metalloproteinases, MMP1 and 10, appear in CSF of subjects with VCID. We are using histopathology to test where these proteins originate.

Methods: We performed immunohistochemistry for MMP1 and 10 on 14 cases +/- AD taken from our archival post-mortem brain bank and from new cases obtained through the New Mexico Office of Medical Investigator. We estimated Braak stage using silver stain or IHC on inferior parietal lobule and/or superior and medial temporal gyrus and middle frontal gyrus. We also examined cerebral vessels for evidence of vascular disease. MMP-staining was performed on sections of forebrain, temporal gyrus and pons.

Results: Anti-MMP1 stained arterial smooth muscle and pericytes. Staining was not visible in venous structures. Capillaries stained only part-way along their length, suggesting that pericytes did not uniformly surround them. MMP1 demonstrated strong staining around arterioles penetrating Virchow's space and in white matter. Anti-MMP10 stained perivascular macrophages, more prevalent in cases with vascular disease. Localized faint neuronal staining not correlating with p-tau was observed in cases selected as pathological controls. In several cases of high Braak score, long neuronal-like processes were stained, with only minimal staining of dystrophic neurites in plaques and none of neurofibrillary tangles.

Conclusions: We propose that expression of MMP10 may be induced in neurons by injury– ischemia or inflammation. Our preliminary conclusions are that MMPs appear in the CSF from vascular origination, that MMP1 is a useful marker for arterial smooth muscle and pericyte integrity, and that MMP10 will inform on perivascular inflammation and neuronal injury in histopathology of VCID and AD.

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Characterization of Parenchymal and Vascular Aβ-42 Deposits in the Cerebellum of Patients with Alzheimer's Disease

G Lopez, S Magaki, C Williams, H Vinters
University of California, Los Angeles

Background: The importance of cerebellar involvement in Alzheimer's disease (AD) is increasingly recognized. However, Aβ deposits in this anatomic compartment are not well characterized, and their clinical significance is largely undefined.

Methods: Cerebellar parenchymal and vascular Aβ-42 deposits were evaluated using immunohistochemistry in 73 cases of pure or mixed AD with an A3 score (by ABC criteria) with respect to localization, morphology, density, and intensity, and clinicopathologically correlated.

Results: Sixty-three of 73 cases demonstrated cerebellar Aβ-42 parenchymal staining (mean age 75.2 years, male/female ratio 0.97) while 10 cases were negative for Aβ-42 (mean age 74.6 years, male/female ratio 1.50). Cases with cerebellar Aβ-42 deposition had a higher proportion with a B3 score (Braak stage V/VI, $p < 0.01$, chi-squared test) compared to Aβ-42-negative cases. Patterns of staining were diffuse in 47 (74.6%) cases, compact without a central dense core in 20 (31.7%), and compact with a central dense core in 10 (15.9%, all corresponding to plaques). Aβ-42 staining was observed in the molecular layer of 53 (84.1%) cases, in the Purkinje cell (PC) layer of 31 (49.2%) cases, and in the internal granular layer of 22 (34.9%) cases. Brains with PC loss had a higher proportion of Aβ-42 PC layer positivity compared to cases without PC loss ($p = 0.02$, chi-squared test). Cerebral amyloid angiopathy was observed in the leptomeninges in 32 (43.8%) cases and in the parenchyma in 14 (19.2%) cases.

Conclusions: Cerebellar Aβ plaques show a unique morphological spectrum. Deposits of Aβ in the cerebellum are seen more often in brains with higher Braak stage, and Aβ-42 immunoreactivity in the PC layer is associated with PC loss.

Characterization of AD pathologic change in the cerebellum may help in understanding its role in the pathogenesis of AD.

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Senile plaque-associated transactive response DNA-binding protein 43 (TDP-43) in Alzheimer's disease: A case report

A Carlos, S Koga, K Josephs, D Dickson
Mayo Clinic

Background: Transactive response DNA-binding protein 43 (TDP-43) pathological inclusions are found in frontotemporal lobar degeneration (FTLD-TDP) and Alzheimer's disease (AD-TDP). While clinically different, TDP-43 inclusions in FTLD-TDP and in AD can have similar morphological characteristics. However, TDP-43 colocalizing with tau and forming "apple-bite" neuronal cytoplasmic inclusions (NCI) or flame-shaped neurofibrillary tangle (NFT)-like inclusions are only found in AD-TDP. Here we describe a case with AD and senile plaque-associated TDP-43.
Methods: N/A
Results: Case Presentation: The patient was a 96-year-old right-handed Caucasian female, who had developed a slowly progressive amnestic syndrome compatible with typical AD at age 82 and was enrolled in the Alzheimer's Disease Neuroimaging Initiative study. Consistent with the old age at onset and long disease duration, limbic-predominant AD was found at autopsy, with high hippocampal yet low cortical NFT counts. Sparse Lewy bodies and Lewy neurites were seen in the amygdala and olfactory bulb. Other pathologies included aging-related tau astrogliopathy and mixed small vessel disease (arteriolosclerosis and amyloid angiopathy) with several cortical microinfarcts. Hippocampal and amygdala sclerosis were present. Immunohistochemistry for phospho-TDP-43 showed NCIs, dystrophic neurites, and rare neuronal intranuclear inclusions, consistent with FTLD-TDP type A. These were frequent in the amygdala, entorhinal cortex, hippocampus, occipitotemporal gyrus, and inferior temporal gyrus, but sparse in the mid-frontal cortex. Additionally, there were TDP-43-immunoreactive inclusions forming plaque-like structures in the molecular layer of the dentate fascia of the hippocampus. The presence of neuritic plaques in the same region was confirmed using thioflavin S fluorescent microscopy and immunohistochemistry for phospho-tau. Double labeling immunofluorescence showed colocalization of TDP-43 and tau within senile plaques.
Conclusions: TDP-43 can colocalize with tau in neuritic plaques in AD which expands the association of TDP-43 and tau in AD beyond neurofibrillary tangles. Given the patient's old age, the clinical correlate of this plaque-associated TDP-43 is unsurprisingly a slowly progressive amnestic syndrome.

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Survey of Neuroanatomic Sampling and Staining Procedures in Alzheimer Disease Research Center Brain Banks

B Dugger[1], J Vizcarra[2], A Teich[3], D Gutman[2],
ADRC Digital Pathology Working Group[4]
[1]University of California, Davis; [2]Emory University; [3]Columbia University; [4]Alzheimer's Disease Research Centers

Background: The collection of post-mortem brain tissue has been a core function of the Alzheimer's Disease Research Center's (ADRCs) network since its inception. Individual brain banks and centers follow detailed protocols to record, store, and manage complex datasets that include clinical data, demographics, and when post-mortem tissue is available, a detailed neuropathological assessment. Since each institution often has specific research foci, there can be significant variability in tissue collection and processing workflows. While published guidelines exist for select diseases, such as those put forth by the NIA-AA, it is of importance to denote the current practices across institutions.
Methods: A survey was developed and sent to brain bank leaders, collecting data on brain region sampling, including anatomic landmarks used, staining (including antibodies used), as well as whole-slide-image scanning hardware.
Results: We distributed this survey to 40 brain banks and obtained a response rate of 95% (38 / 40). Most brain banks followed guidelines defined by the NIA-AA, having H&E staining in all recommended regions and targeted region-based amyloid beta, tau, and alpha-synuclein immunohistochemical staining. However, sampling consistency varied related to key anatomic landmarks/locations in select regions, such as the striatum, periventricular white matter, and parietal cortex.
Conclusions: This study highlights the diversity and similarities amongst brain banks and discusses considerations when amalgamating data/samples across multiple centers. This survey aids in establishing benchmarks to enhance dialogues on divergences in workflows in a feasible way.

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Neurovascular complications of anti-amyloid-β immunotherapy: Report of a case

P Jamshidi, E Shanes, M McCord, M Flanagan, R Castellani
Northwestern Memorial Hospital

Background: Host responses to anti-amyloid-β (Aβ) antibody therapy are evident in neuroimaging changes and clinical symptoms in a subset of clinical trial subjects receiving such therapy. The pathological basis for the imaging changes and clinical symptoms is not known, nor is the precise mechanism of Aβ clearing.
Methods: We report the autopsy findings in a 65-year-old woman who received three open label infusions of the experimental anti-Aβ drug lecanemab. About one month after the first lecanemab infusion, she presented with acute stroke and died four days later.
Results: Neuropathological examination demonstrated predominantly histiocytic vasculitis encompassing fibrinoid necrosis of blood vessel walls involved by cerebral amyloid angiopathy (CAA). Fragmentation and phagocytosis of vascular Aβ were present throughout the cerebral cortex. Phagocytosis of parenchymal Aβ plaques was noted. Apparent evidence of Aβ and possibly phosphorylated tau "clearing" was also noted.
Conclusions: The findings overall suggest that anti-Aβ treatment stimulated a host response to Aβ, i.e., target engagement. The findings also provide evidence that amyloid-related imaging abnormalities (ARIA) in some cases are indicative of an Aβ phagocytosis syndrome within cerebral vasculature and parenchymal brain tissue.

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A Unique Case of Globular Glial Tauopathy Type I and Alzheimer Disease Neuropathological Changes

Y Liu, M Miyakawa-Liu, J Nirschl, K Vallejo, S Sha, I Cobos
Stanford University

Background: Tauopathies, a group of clinically and pathologically heterogeneous neurodegenerative disorders, are characterized by the presence of microtubule-associated tau protein aggregates in neurons and glia. Globular glial tauopathy (GGT) is a recently identified tauopathy characterized by widespread globular glial inclusions (GGIs) and belongs to a group of four-repeat (4R) tauopathies. In addition to the diverse set of clinicopathological presentations, the rarity of GGTs also significantly impedes accurate recognition and diagnosis.
Methods: In this case study, we provide postmortem neuropathological characterization of an 86-year-old female with Type I GGT with tau aggregates predominantly in white matter oligodendrocytes. The patient's clinical diagnosis was frontotemporal dementia (FTD) with semantic variant primary progressive aphasia (svPPA) with presumed TDP-43 pathology, although Alzheimer disease (AD) was on the differential. The patient demonstrated progressive behavioral and cognitive decline for approximately 11 years prior to death. Her mental status exam was characterized by the presence of flat affect and intermittent eye contact with speech paucity and automatic language use. Atypical features of her clinical presentation included advanced age of onset and hippocampal involvement with memory impairment.
Results: On autopsy, the brain had fronto-temporal lobar atrophy with severe medial temporal lobe degeneration. Histologic sections with tau immunohistochemistry (AT8) demonstrated accumulation of abundant globular oligodendroglial inclusions (GOI) in the white matter of the frontal lobe and superior and middle temporal gyri. Scattered globular astroglial inclusions (GAI), rare coiled bodies, and rare neurofibrillary tangles were also seen. Additionally, amyloid plaques in the neocortex, hippocampus, basal ganglia, and brainstem were identified, consistent with the presence of Alzheimer disease neuropathological changes. Stainings for TDP-43 and alpha-synuclein were negative.
Conclusions: Overall, the abundant accumulation of GOI in the frontotemporal white matter is supportive of a diagnosis of GGT Type I with AD co-pathology.

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Novel Increase of N- and O-linked Sialylation in Alzheimer's Disease

C Fastenau[1], J Wickline[2], S Smith[2], S Hopp[2], K Bieniek[2]
[1]University of Texas Health Science Center San Antonio; [2]UT Health Science Center San Antonio

Background: Research shows evidence of increased sialylation in post-mortem Alzheimer's Disease (AD) cases. Sialylation is a form of glycosylation with the attachment of terminal sialic acid (SA) to glycoproteins and lipids. There are two major forms of sialylation, N- and O-linked SA, that partially regulate microglia cellular interactions. Yet it is unclear how these forms of sialylation contribute to AD pathology and modify disease progression. Our previous work in 5XFAD mouse model of amyloid pathology demonstrate strong α-2,6 microglia sialylation in the plaque microenvironment. We hypothesize specific microglia sialylation patterns early in disease progression contribute to pathological burden and dampen immune response.
Methods: We utilized tissue from 10 post-mortem late-onset AD cases with an average age of 72.2 years. We specifically analyzed frontal, hippocampal, and cerebellar regions. To Identify N-linked SA bonds, we focused on α-2,6 SA which was recognized by a plant-derived lectin. Aβ plaques, neurofibrillary tangles, microglia, and α-2,6 SA were labelled with immunofluorescent markers. Confocal microscopy images were analyzed with Image J FIJI. To identify O-linked SA bonds, Alcian Blue and Periodic Acid Schiff stains were used in novel ways to differentiate subsets of neutral and charged O-linked SA bonds. Samples were imaged on a Aperio microscope slide scanner and analyzed on Imagescope.
Results: These AD cases share similar α-2,6 microglia sialylation patterns proximal to plaques. Yet, there was no significant correlation of between tau and α-2,6 SA ($r = -0.143$; $p < 0.0001$) in human AD compared to microglia sialylation ($r = 0.279$). Intriguingly, these cases also have unique patterns of O-linked sialylation proximal to Aβ plaques.
Conclusions: Moving forward, we plan to 1) determine the correlation between Aβ plaques and α-2,6 sialylation compared to microglia sialylation in Human AD and 2) investigate subsets of plaque associated microglia to determine what forms of O-linked SA may reduce clearance of debris by microglia.

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Neuropathology of the Jalisco A431E PSEN1 Mutation in Comparison to Other PSEN1 Mutations in Autosomal Dominant Alzheimer's Disease

M Estrella[1], K Hird[1], V Goodwill[1], D Pizzo[1], D Galasko[1], P Gopal[2], S Guzman[3], D Hawes[4], J Ringman[4], C Church[4], A Hiniker[1]
[1]University of California San Diego; [2]Yale University; [3]University of Colorado; [4]University of Southern California

Background: PSEN1 mutations cause up to 50% of Autosomal Dominant Alzheimer's disease (ADAD) cases. Unique to a subset of PSEN1 ADAD cases, including the Jalisco A431E mutation, are symptoms of very early spastic paraparesis frequently preceding onset of cognitive decline. Importantly, the neuropathology of the Jalisco A431E PSEN1 mutation, thought to be one of the most common drivers of ADAD in persons of Mexican origin, has not yet been described.
Methods: In this study, we aimed to characterize the neuropathology of the Jalisco A431E PSEN1 mutation, particularly related to the development of spastic paraparesis and in comparison to other PSEN1 mutations. Across two institutions (UCSD, USC), we identified a cohort of seven ADAD patients with the Jalisco A431E PSEN1 mutation and nine ADAD patients with other PSEN1 mutations (two N135D, one F388S, two L435F, three T245P and one A260V). Ongoing work compares standard semi-quantitative neuropathologic data (H&E, Gallyas, p-tau, Abeta, a-synuclein, TDP43, in accordance with NACC Version 11 guidelines), as well as white matter loss and vascular disease.
Results: The cohort consisted of eight males and eight females, with an average age at death of 47.2 years, and an average disease duration of nine years. Nine patients presented with spastic paraparesis, including all seven A431E mutation carriers, in addition to the F388S and A260V mutation carriers. All but two of the PSEN1 mutation carriers showed extreme amyloid plaque and tau burden with a Thal Phase 5 (A3), Braak stage VI (B3), and frequent neuritic plaques (CERAD C3). The characteristic "cotton wool" amyloid plaque morphology was often evident.
Conclusions: Ongoing work will use digital pathology, quantitative assessments, and detailed pathologic analysis, to characterize the neuropathology of the Jalisco PSEN1 mutation. Additionally, we hope to identify trends in neuroanatomic distribution of neuropathology that may account for unique clinical movement abnormalities observed in many of these patients.

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High-Throughput Digital Quantification of Alzheimer's Disease Pathology in Large-Scale Autopsy Studies

A Kapasi[1], J Poirer[2], A Hedayat[2], A Scherlek[2], J Gibbons[2], S Leurgans[2], J Schneider[2]
[1]Rush Alzheimer's Disease Center; [2]Rush Alzheimer's Disease Center

Background: The most recent NIA-AA guidelines for a neuropathologic diagnosis of Alzheimer's disease (AD) relies upon traditional practices of semi-quantitative assessment and topographical staging of beta-amyloid and tau-tangle pathologies. High-throughput digital pathology offers considerable advantages; deeper phenotyping of brain tissue specimens, unbiased, robust, and reproducible data, quantification of pathology across whole region of interest, and harmonization of data across centers.
Methods: We used brain tissue from five clinical-pathologic cohort studies; the Religious Orders Study, the Rush Memory and Aging Project, the Minority Aging Research Study, Clinical Core, and Latino Core, to 1) create a digital library of whole slide images (WSI), and 2) develop automated methods to aid identification of AD pathology.
Results: Digital algorithms for the quantification of beta-amyloid (n = 412 WSI) and tau-tangles (n = 639 WSI) showed excellent correlations with manual pathology data (rs = 0.83 - 0.94). Data were highly robust and reproducible across different magnification parameters and repeated scans. Using digital measures, we show that beta-amyloid and tau-tangles across multiple brain regions reproduce established patterns of correlations, as well as when samples are stratified across clinical diagnoses. Finally, we use statistical approaches to harmonize newly generated digital measures with historical measures across multiple large-scale autopsy studies.
Conclusions: Digital pathology is a powerful tool that provides high-resolution neuropathologic information for diagnostics and for basic and translational research.

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Alzheimer's Disease with Substantia Nigra Neurodegeneration

D Ono[1], H Sekiya[2], M Murray[2], D Dickson[2], S Koga[2]
[1]Department of Neuroscience, Mayo Clinic; [2]Mayo Clinic

Background: Alzheimer's disease (AD) is neuropathologically heterogeneous and has been classified into three main corticolimbic subtypes – hippocampal sparing, typical, and limbic predominant – based on the severity and distribution of neurofibrillary tangles. Neurodegeneration in the substantia nigra is observed in a subset of AD cases, but its etiology remains to be determined. The present study aimed to elucidate neuropathologic features of AD with nigral neurodegeneration (AD-SN).
Methods: We studied pathologically-confirmed AD from the Mayo Clinic brain bank. Cases with comorbid Lewy body pathology, progressive supranuclear palsy, corticobasal degeneration, multiple system atrophy, frontotemporal lobar degeneration, or cerebrovascular disease were excluded. The degree of neuronal loss in the substantia nigra was semi-quantitatively assessed on hematoxylin and eosin stained sections on a four-point scale: normal, mild, moderate, and severe. Cases with moderate or severe neuronal loss were classified as AD-SN.
Results: We identified 119 AD-SN (20%) in 591 AD cases without comorbid pathology. AD-SN cases had higher Braak

neurofibrillary tangle stage (5.7 vs 5.5; P = 0.001), higher frequency of TDP-43 pathology (36% vs 21%; P = 0.001), and longer disease duration (10.0 vs 8.3 years; P = 0.001) than AD without nigral neurodegeneration. There were no significant differences in demographic or other pathologic features, including age at death (76.5 vs 76.8 years), sex (45.4% vs 48.9% male), Thal amyloid phase (4.7 vs 4.7), and AD neuropathologic subtypes (hippocampal sparing: 23% vs 25%, typical: 65% vs 65%, limbic: 12% vs 10%).

Conclusions: Our findings confirmed that a subset of AD cases had neuronal loss in the substantia nigra that is not associated with concurrent Lewy-related pathology. AD-SN cases are characterized by longer disease duration and higher frequency of TDP-43 pathology.

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Correlation of Presynaptic and Postsynaptic Proteins with Pathology in Alzheimer's Disease

G Serrano, T Beach, J Walker
Banner Health

Background: Synaptic transmission is essential for nervous system function and its dysfunction is a known major contributor to dementia. The correlation between synaptic loss and Alzheimer's disease dementia (ADD) was established in the late 1980s using electron microscopy (EM) techniques. These methods are precise but are limited the laborious tissue processing required and by their practical restriction to extremely small tissue samples. In the 1990s, immunochemical quantification became possible and confirmed 30-50% neocortical synaptic protein losses in ADD, but there has not yet been a comprehensive profiling of different synaptic proteins in different brain regions in ADD.

Methods: In this study we quantified densities of two synaptic proteins, the presynaptic protein SNAP25 and the postsynaptic protein PSD95 using enzyme-linked immunosorbent assays (ELISA). Cingulate, hippocampus, frontal, visual and entorhinal cortex were dissected for protein extraction from non-demented controls (ND, n = 50) and ADD subjects (n = 50). Cases were selected by a database search of the Arizona Study of Aging and Neurodegenerative Disorders (AZSAND) and Brain and Body Donation Program (BBDP).

Results: SNAP25 is significantly reduced in ADD when compared to controls in frontal and visual cortex and cingulate, while hippocampus showed non-statistically significant reduction and entorhinal cortex showed similar protein concentration. In contrast, all brain areas tested showed lower PSD95 concentration in ADD when compared to non-demented controls, except hippocampus that only showed a non-statistically significant reduction. SNAP25 and PSD95 concentrations significantly correlated with neurofibrillary tangle pathology, plaques and MSSEs scores.

Conclusions: Our results suggest that synaptic transmission in the entorhinal cortex of ADD patients is severely affected, most probably because this brain region is one of the earliest affected areas and by the time of clinically-manifest dementia has very high densities of neurofibrillary tangles. The entorhinal area is the main interface between the hippocampus and neocortex, making this area crucial for memory formation and consolidation.

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Tau Distribution and Cognitive Impairment in Posterior Cortical Atrophy and Early Onset Alzheimer's Disease

D Smirnov, S Dickinson, M Estrella, V Goodwill, A Headley, D Pizzo, D Galasko, D Salmon, A Hiniker
UC San Diego

Background: Early-onset sporadic Alzheimer's disease (AD) patients often exhibit non-memory symptoms attributed to relatively greater neocortical than hippocampal tau neurofibrillary tangle (NFT) density. Posterior cortical atrophy (PCA) is one variant of AD, clinically defined by visuospatial deficits at disease onset.

Methods: This study investigates the difference in tau neurofibrillary tangle (NFT) density in the brains of early-onset sporadic AD patients with and without a clinical PCA diagnosis. Fifty patients with early onset (age < 65), non-familial, and autopsy-confirmed AD were examined from the UCSD Alzheimer's Disease Research Center, including 12 with a PCA diagnosis. NFT density was measured in the hippocampus (HP), midfrontal cortex (MF), and occipital cortex (OC).

Results: PCA and non-PCA patients did not significantly differ in age (63.35±4.59 years at death) or sex (40% female). The APOE ε4 allele was much less frequent in PCA (p = 0.002). Though PCA patients were less impaired on the MMSE (p = 0.01), they performed comparably to non-PCA patients on the CDR sum-of-boxes. While the absolute occipital cortex NFT density did not differ between the two groups, PCA patients had greater OC/HP (p = 0.04) and OC/MF (p = 0.008) NFT ratios. Lower visuospatial performance was associated with increased OC NFT density (p = 0.006), as well as higher OC/HP (p = 0.007) and OC/MF NFT ratios (p = 0.03), even when restricting to non PCA participants. In contrast, lower memory performance was associated with increased HP NFT density (p = 0.02).

Conclusions: Despite differences in the predominance of early visuospatial symptoms, PCA and non-PCA patients did not differ in absolute OC tangle density, with some of the highest densities in non-PCA patients. However, the degree of visuospatial impairment was associated with the OC/HP NFT ratio across both groups. These findings suggest that the prominence of visuospatial impairment at onset, a hallmark of PCA,

may be masked by the degree of HP pathology and memory impairment in non-PCA patients.

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Automated Segmentation of Blood Vessels Walls and Lumens on Digitized H&E Stained Brain Tissues Using Deep Learning

J Lou[1], P Chang[2], K Nava[3], C Chantaduly[4], H Wang[3], E Monuki[5], H Vinters[6], S Magaki[6], V Patel[7], W Christopher[8], D Harvey[7], M Keiser[8], B Dugger[7]
[1]Department of Pathology, University of California, Irvine,-School of Medicine; [2]University of California Irvine School of Medicine; [3]University of California, Davis; [4]University of California, Irvine; [5]University of California, Irvine,School of Medicine; [6]David Geffen School of Medicine at UCLA; [7]University of California, Davis, School of Medicine; [8]University of California, San Francisco

Background: Automated morphometric analysis of vascular changes through deep learning using standard histological stains may facilitate a greater understanding of vasculopathy in various neurologic disorders including Alzheimer disease.
Methods: This study builds upon our previously published method that detected blood vessels within 256x256 pixels patches. Upon visual inspection, blood vessel(s) within these patches were often located on patch edges, with some vessels cropped, which was hypothesized to reduce segmentation performance. In this study, a second algorithm is added to produce new patches centered on the detected blood vessel(s), for each of which, pixel-level masks were generated for the vessel wall and vessel lumen. This study includes 8 whole slide images generated from H&E stained slides of human brain frontal and occipital white matter in 4 participants (2 male, 2 female; mean age 73, standard deviation 20) identified from two institutions (UC-Davis and UC Irvine). Data augmentation through random rotation, translation, and flip was performed. A modified Unet with 77 layers and 689 198 trainable parameters exclusive of 117 749 120 ImageNet pretrained non-trainable parameters from a frozen EfficientNetV2L backbone was trained and tested on 108 blood vessel(s) centered patches and corresponding annotation masks.
Results: The 12-fold cross validation Dice index for the segmentation of vessels walls and lumens combined was 0.66; for vessels walls only 0.66; and vessels lumens only 0.64. The Pearson correlation coefficient for vessels walls and lumens combined was 0.76 ($p = 0.005$) for the current study and 0.55 ($p < 0.001$) for the previously published method.
Conclusions: This pilot study demonstrates a proof of concept that deep learning algorithms can detect and semantically segment walls and lumens of blood vessels with promising accuracy and that centering patches on blood vessels may improve algorithm performance. Further optimization is warranted to produce high level accuracy for the automated morphometric analysis of vasculopathy.

SATURDAY POSTERS: Demyelinating and Inflammatory

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Rare Case of Marchiafava-Bignami Disease in a Patient with Insulin-Dependent Diabetes Mellitus and No History of Alcohol Use

M Gubbiotti, A Banihashemi
Hospital of the University of Pennsylvania

Background: A 55 year-old female patient with a past medical history of end-stage renal disease (ESRD) on hemodialysis, hypertension, and poorly-controlled insulin-dependent diabetes (blood glucose on admission: 413 mg/dL, reference: 70-99 mg/dL) presented to the hospital for incision and drainage of a gluteal abscess. Her stay was complicated by bouts of altered mental status including left-sided visual and sensory neglect, aphasia, left facial droop, and left arm drift.
Methods: Clinical work-up revealed thiamine deficiency (50 nmol/L, reference: 70-180 nmol/L). Brain magnetic resonance imaging (MRI) showed regions of diffusion restriction in the body of the corpus callosum with patchy enhancement. Additionally, diffuse restriction was noted in bilateral cerebral peduncles, ventral pons, and medullary pyramids. Her symptoms moderately improved with thiamine repletion. Due to complications from sepsis, she died nearly four months from day of admission.
Results: Autopsy was performed revealing multiple dark, brown puncta within the anterior genu, body, and splenium of the corpus callosum, bilaterally. Otherwise, the brain findings were within normal limits. Histologic examination revealed extensive patchy demyelination of the aforementioned regions of the corpus callosum with associated macrophages and microhemorrhages. These regions of demyelination were highlighted by absence of Luxol Fast Blue staining and the presence of CD68+ macrophages. Luxol Fast Blue stain also highlighted engulfed myelin by macrophages. To a lesser extent, additional regions of demyelination were seen in the right and left crus of the midbrain, pons, and medulla.
Conclusions: Taken together with the clinical and radiologic findings, a diagnosis of Marchiafava-Bignami was rendered. Only few reports of this disease have been documented in the literature in patients with uncontrolled diabetes without significant alcohol use: this case would be the fifth. The mechanism for this process is still unknown, but posited to involve a hyperosmolar state leading to alterations in osmotic pressure.

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A case of crystal-storing histiocytosis involving the medial temporal lobe

S Neill[1], P Agarwal[1], D Larson[2], B Morales-Vargas[1], M Schniederjan[1], L Zhang[1]
[1]Emory University School of Medicine; [2]Mayo Clinic College of Medicine and Science

Background: Crystal-storing histiocytosis (CSH) is an uncommon disorder that can involve a variety of tissue sites including, rarely, the central nervous system (CNS). In most cases, CSH is associated with an underlying hematolymphoid neoplasm, especially tumors that can express monoclonal immunoglobulins. In exceptional cases CSH has been reported in association with infectious or inflammatory conditions; of note, several examples of CNS CSH have been associated with non-neoplastic diseases. Histologically, the disorder is characterized by a proliferation of histiocytes containing intracytoplasmic accumulations of immunoglobulin that aggregate into crystalline structures.
Methods: We report the case of a 24-year-old man who presented with a several-week history of headache. MRI disclosed an ill-defined FLAIR signal abnormality with patchy enhancement in the medial left temporal lobe; additional smaller similar areas were observed in the right temporal lobe and sublentiform region. A surgical resection of the left temporal lesion was pursued.
Results: Histologic examination revealed cortex and white matter involved by a dense histiocytic infiltrate present in a background of perivascular and parenchymal lymphoplasmacytic inflammation. The histiocytes contained innumerable intracytoplasmic eosinophilic crystalline deposits exhibiting a variety of appearances, including granular, globular, and rod-shaped forms. An extensive histochemical, immunohistochemical, molecular, and biochemical workup revealed no evidence of infection, storage disorder, or histiocytic neoplasia; a clonal IgH/IgK rearrangement was detected by PCR and the histiocytes were shown by mass spectrometry to contain kappa immunoglobulin light chains and mu immunoglobulin heavy chains. Definitive histologic and molecular evidence of a lymphoproliferative disorder has not been detected.
Conclusions: CSH is a rare, morphologically striking phenomenon within the CNS associated with a variety of underlying disorders; knowledge of its existence and key features is important for the practicing surgical neuropathologist.

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Concurrent Progressive Multifocal Leukoencephalopathy and CNS Lymphoma in the Setting of Untreated HIV Infection

R Alfattal, B Shine, M Felicella
University of Texas Medical Branch - Galveston

Background: Immune-dysregulation and suppression are associated with the development of lymphoproliferative disease. Immunodeficiency-associated CNS lymphoma (IACNSL) is an aggressive neoplasm that often presents at a late clinical stage.
Methods: We report an unusual case of concurrent IACNSL and progressive multifocal leukoencephalopathy (PML) in immunosuppressed individual, who has experienced an indolent disease course following limited treatment with antiretroviral therapy and a single dose of rituximab.
Results: A 55-year-old Black male with previously untreated HIV infection presented with a 40-pound weight loss, nausea, vomiting, dysmetria, ataxia, and progressive bilateral lower extremity weakness. Laboratory evaluation showed an HIV viral load of 66,797 copies/mL and CD4 count of 51 cells/uL. Antiretroviral therapy was initiated, and he was diagnosed clinically with Miller-Fisher syndrome; his symptoms improved with intravenous immunoglobulin therapy. He was stabilized and discharged but was readmitted one month later for intractable vomiting and worsening neurologic symptoms. MR imaging showed extensive confluent T2/FLAIR hyperintensity involving the pons and cerebellum with faint enhancement. CSF analysis showed elevated protein, normal glucose, and an abnormal lambda-predominant B-cell population on flow cytometry; EBV and JC viruses were detected by PCR. He received one dose of rituximab; however, repeat MRI two weeks later showed interval increase in T2/FLAIR hyperintensity with minimal enhancement or mass effect. A stereotactic brain biopsy revealed classic features of PML and an atypical perivascular B-cell infiltrate. B-cell clonality was confirmed by molecular studies, supporting the diagnosis of EBV-related B-cell lymphoma. The patient did not receive additional chemotherapy or radiation and both his lymphoproliferative disease and PML have remained stable for 27 months on only antiretroviral therapy.
Conclusions: Although favorable prognosis in IACNSL has been correlated with intensive treatment with immunomodulators, prognostic markers are not well-established. This case represents an unusually indolent course of two concomitant aggressive diseases treated only with antiretroviral therapy.

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Neurosarcoidosis: Challenges from a non-endemic region

B Kleinschmidt-DeMasters
University of Colorado Anschutz Medical Campus

Background: Neurosarcoidosis (NS) is a challenging diagnosis, especially in low-prevalence regions where it is seldom high amongst differential considerations. In the United States, the highest incidence is the Midwest and Northeast, with a 3X greater incidence in African-Americans. NS is well-known to mimic meningeal carcinomatosis, autoimmune or infectious pachymeningitis, or tuberculosis, but is even more likely to be unsuspected if systemic signs of sarcoidosis are lacking or if dural-based or sellar masses are the first presentation of disease. Further challenging is when NS presents as myelopathy.
Methods: Search of Colorado departmental database and personal files, 2005-2021, for NS cases, focusing on surgical/cytology cases where CNS specimen represented the first confirmed diagnosis of sarcoidosis and on autopsy cases in which sarcoidosis/NS caused myelopathy and demise.
Results: 18 surgical/cytology cases were identified (7M:11F, 13-66 years), in 9 of which CNS specimen had been first diagnosis of sarcoidosis. Clinical follow-up confirmed sarcoidosis and excluded other conditions. One patient initially thought to have sarcoidosis based on a muscle biopsy containing classic non-necrotizing compact granulomas, was excluded after diagnosis of Wong-type hypomyopathic dermatomyositis (TIF1-

gamma +), with work up leading to diagnosis of stage IA bladder carcinoma. The remaining 18 surgical/cytology cases included 7 Caucasians, 6 African-Americans, 4 Hispanics, 1 Pacific-Islander. 2/18 had dural-based masses, one with pure NS as the cause of her meningioma-like lesions and the second with meningioma intimately admixed with non-necrotizing granulomas of NS. 2 autopsy cases were from 38- and 43-year old women, both with cauda equina syndrome/ progressive weakness as first presentation, and extensive spinal cord/nerve root sarcoidosis and cardiac involvement contributing to demise. NS/sarcoidosis was unsuspected premortem in the former.
Conclusions: NS involves unusually diverse anatomical regions, occasionally co-exists with tumors, and requires clinical follow-up for firm diagnosis. NS yields diagnostic challenges especially in low incidence areas.

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Acute Necrotizing Encephalopathy with Extensive Spinal Cord Involvement

M Priddy-Arrington, A Shepler
Atrium Health Wake Forest Baptist

Background: Acute necrotizing encephalopathy (ANE) is a rare disease that presents with episodes of acute encephalopathy preceded by a febrile (often viral) illness and is thought to be an immune-mediated process. Symmetric, multifocal brain lesions characteristically involve the bilateral thalami and brainstem. Spinal cord involvement has only rarely been reported. Some cases are associated with a mutation in the gene encoding RAN Binding Protein 2 (RANBP2), which predisposes to recurrent episodes.
Methods: A 5-year-old male with a history of acute necrotizing encephalopathy (beginning at 10 months of age) with pathogenic RANBP2 mutation and secondary epilepsy presented with altered mental status and seizures following high fevers and influenza A infection. Imaging showed a small hemorrhagic lesion of low diffusivity in the left cerebellar hemisphere and sequelae of prior ANE episodes. His mental status deteriorated with development of diffuse cerebral and cerebellar edema, hydrocephalus, and tonsillar herniation. An external ventricular drain was placed; however, he continued to decline and was pronounced five days after admission.
Results: At autopsy, the brain showed severe diffuse cerebral edema with softened and cavitated areas within the thalami, claustra, external capsules, lentiform nuclei, and pons, consistent with older lesions. Microscopically, these areas showed ferruginated neurons and gliosis. More recent lesions were present in the brainstem and cerebellum, with soft hemorrhagic foci of the tegmentum of the midbrain and scattered pontine and cerebellar petechial hemorrhages. Histologically, these areas showed parenchymal vacuolation with acute neuronal necrosis and petechial hemorrhages. There was no associated intraparenchymal inflammation or evidence of a vasculitis. Grossly, the entire length of the spinal cord was severely edematous. Sectioning revealed intraparenchymal hemorrhages and duskiness of the thoracic spinal cord, which microscopically showed parenchymal vacuolation with acute petechial hemorrhages and scattered necrotic neurons without inflammation.
Conclusions: The extensive spinal cord involvement makes this an unusual case of a rare disease.

SATURDAY POSTERS: Developmental/Pediatric

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Cellular proliferation in dorsal root ganglia of Friedreich ataxia

A Koeppen[1], J Mazurkiewicz[1], P Feustel[1], S Pelech[2], C Sutter[3], S Ahmad[4], H Khan[4]
[1]Albany Medical College; [2]Kinexus Bioinformatics Corp; [3]Kinexus Bioinformatics Corp; [4]VA Medical Center

Background: Friedreich ataxia (FA) causes destruction of large and small nerve cells in dorsal root ganglia (DRG). Hypercellularity in DRG includes disorganization and proliferation of satellite cells; invasion by IBA-1 reactive monocytes, neuronophagia; and formation of residual nodules.
Methods: The hypothesis in this work is that frataxin deficiency, the underlying mutation in FA, causes downstream changes in DRG proteins.
Results: Antibody microarray of DRG lysates with 800 validated antibodies that target cell signaling proteins revealed prominent up-regulation of the stem-cell factor (SCF) receptor tyrosine kinase KIT phosphorylated at Tyr 936. In its bioactive form upon engagement with SCF, KIT undergoes dimerization and phosphorylation at Tyr 936. By double-label laser scanning confocal immunofluorescence, KIT-pY936 is located in nuclei and colocalizes with cytoplasmic SCF.
Conclusions: KIT is an oncogenic protein with prominent roles in hematopoiesis including induction of mast cell proliferation. The reason for its significant augmentation in lysates of DRG in FA remains elusive.

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A rare ventricular lesion in an infant

D Xu, C Kent, R Robison, R Raghavan
Loma Linda University

Background: Primary intraventricular vascular lesions are rare
Methods: Histopathologic and immunohistochemical analysis with chart review
Results: The lesion described came from a 4 month old girl born at 25wks and transferred for further management with moderate patent ductus arteriosus s/p closure, history of bacteremia with presumed endocarditis, and gastro-intestinal bleeding with perforated necrotizing entero-colitis resulting in extensive small bowel resection. A head ultrasound showed a hyperechoic and vascular lesion in the fourth ventricle measuring up to 1.7 cm with a follow up MRI confirming a lobulated fourth ventricular mass extending to the left Foramen of

Luschka. Of note, this was the mother's first child, with pregnancy complicated by substance abuse, and smoking 1 cigarette pack per day. The lesion consisted of a circumscribed proliferation of closely packed, and congested capillary-sized vessels, separated by thin fibrous stroma. Choroid plexus epithelium was seen adherent to the outer edges of the lesion. Mitotic activity was inconspicuous, and there was no necrosis or other features of malignancy, but Ki67 labeling indices were mildly elevated. No stromal cells or features of a hemangioblastoma were seen, and no Inhibin expression was present on immunostaining. No glioneuronal or ependymal components were present.

Conclusions: The findings were consistent with a Choroid Plexus angioma. Only a single other identical case has been reported in the English literature in this age group (see Ref), and in this location, but that report lacked pathologic details. The etiopathogenesis of this rare entity is unknown, but a full recovery is anticipated, given the presumed benign nature of the lesion. Ref: Labeodan OA. Pediatr Neurosurg. 2004 May-Jun;40(3):153-4

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Neuropathology of a case of alternating hemiplegia of childhood associated with E815K variant of the ATP1A3 gene

T Sano, Y Nakayama, O Kawano, Y Motohashi, N Sumitomo, E Takeshita, T Saito, H Komaki, E Nakagawa, M Mizutani, M Sasaki, M Takao
National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan

Background: Alternating hemiplegia of childhood (AHC) is characterized by recurrent hemiplegic episodes, abnormal ocular movements, involuntary movements, hypotonia, and seizures that begin during infancy. The genetic basis are variants in the ATP1A3 gene. The E815K variant is associated with the most severe AHC phenotype.

Methods: We present an autopsy case report of 41 years female. A woman with no family history of neuromuscular disease developed abnormal eye movements and tonic and convulsive seizures during the neonatal period. At one year of age, she had recurrent hemiplegic episodes. Hypotonia was observed at 4 years old, and she was clinically diagnosed with AHC. At 6 years of age, she achieved ambulation with support at the peak of her development, which was followed by deterioration. Since her teens, multiple (>4) antiepileptic drugs were prescribed for status epilepticus. At 16 years of age, she started to show respiratory paralysis and required tube-feeding. At 18, she was bedridden. At 33, ATP1A3 gene analysis identified Glu815Lys (E815K) variant. By 39 years of age, she needed constant mechanical ventilation. She died from pneumonia at 41.

Results: Brain weight was 762 g. The cerebrum and cerebellum showed severe atrophy. The posterior columns of the spinal cord showed severe degeneration. Severe loss of optic nerve fibers was noted. There was marked atrophy of the hippocampus. Severe loss of Purkinje and granular cells with shrinkage of the molecular layer and degeneration of the dentate nucleus were observed. The thalamus, globus pallidus, and subthalamic nucleus showed severe neuronal loss and gliosis.

Conclusions: Autopsy revealed extensive degeneration of the central nervous system, which corresponds to the variable clinical presentations of AHC. Yet pathology was more extensively distributed (e.g., within the cerebellum, spinal cord, and optic nerves) than expected from the features of AHC in the patient.

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Case Report: Pontocerebellar Hypoplasia Type 6

S Ali[1], M Reyes-Múgica[2], A Panigrahy[3], J Kofler[4]
[1]Department of Pathology, University of Pittsburgh Medical Center; [2]Childrens Hospital of Pittsburgh; [3]Children's Hospital of Pittsburgh; [4]University of Pittsburgh Medical Center

Background: Pontocerebellar hypoplasia type 6 (PCH6) is a rare autosomal recessive condition associated with homozygous or compound heterozygous mutations in the RARS2 gene, encoding mitochondrial arginyl tRNA synthetase. The phenotypic spectrum of this disease is broad, making diagnosis of this entity challenging. While pontocerebellar hypoplasia is seen in some cases, others show progressive atrophy of infra- and supratentorial structures.

Methods: In this report, we describe a case of pontocerebellar hypoplasia type 6 caused by a previously known pathogenic frame shift mutation, c. 452_453insC, and a previously described missense mutation, c. 1544A>G (p. D515G). The female patient presented with lactic acidosis, hypotonia, and prolonged epileptic encephalopathy in the neonatal period. Early neuroimaging identified microcephaly but was otherwise unremarkable. MRI at 2 months showed cytotoxic cortical edema, with subsequent imaging at 4 and 5 months demonstrating progressive volume loss. She exhibited global developmental delay with intractable seizures, and she died at nearly 3 years of age from status epilepticus.

Results: Neuropathological examination demonstrated cerebellar neuronal loss, involving all three cortical layers, and white matter rarefaction and gliosis. The cerebral cortex exhibited a widespread pattern of laminar degeneration with prominent reactive changes and capillary proliferation. There was significant variability in severity of these changes, which did not show an association with vascular territories. Other findings included volume loss of several white matter fiber tracts and an atypical hippocampal sclerosis pattern with severe neuronal loss in the dentate gyrus.

Conclusions: The neuropathologic findings are suggestive of a predominantly neurodegenerative process, likely related to the patient's intractable epilepsy and underlying metabolic abnormalities. This progression of disease is consistent with recent suggestions for considering PCH6 a mitochondrial encepha-

lopathy with progressive global atrophy, in which supratentorial atrophy may be the predominant finding and pontocerebellar hypoplasia may or may not be present.

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Characterization of Pediatric Metastases to the Central Nervous System: A Single Institution Review

J Newman, N Leelatian, J Liang
Vanderbilt University Medical Center

Background: Central nervous system (CNS) metastasis represents a small portion of pediatric CNS neoplasms. Given the limited data, characterization of demographics, clinical course, and mechanism of CNS spread remains to be elucidated.
Methods: Archival institutional pathology records between 1999-2022 were searched for patients 21 years old and younger with CNS metastases (50 cases retrieved). CNS metastasis was defined as involvement of brain or spinal cord parenchyma, dura, leptomeninges, cranial nerve, or cerebrospinal fluid. We documented tumor type and location, metastasis location, method of invasion (direct extension vs. distant metastasis), time to CNS involvement, and outcomes.
Results: Our cohort consists of 33 males and 17 females. The median age at the time of CNS metastasis was 104±78 months. The most common tumor types were neuroblastoma (13, 26%), retinoblastoma (7, 14%), and osteosarcoma (6, 12%). Retroperitoneum (16, 32%) and extremities (8, 16%) were the most common sites of origin. Extrarenal rhabdoid tumor (1, 12 months) and neuroblastoma (13, 28±35 months) had the youngest age at the time of CNS involvement. Melanoma (2, 185±77 months) and inflammatory myofibroblastic tumor (1, 222 months) were most common in older patients. Time to CNS metastasis varied (2±18 months); six tumors demonstrated CNS involvement at initial diagnosis (alveolar rhabdomyosarcoma, malignant germ cell tumor, myeloid sarcoma, neuroblastoma, retinoblastoma, and desmoplastic small round cell tumor). Most tumors reached the CNS through distant metastasis (34, 68%). Dura/leptomeninges (22, 44%) was the most common initial site of CNS metastasis; 3 cases (14%) subsequently developed brain involvement. One neuroblastoma patient showed simultaneous spinal dura and brain parenchyma involvement 16 months after diagnosis. In our cohort, osteosarcoma portends the shortest survival following CNS metastasis (2±1.9 months).
Conclusions: This study highlights the diverse mechanisms and locations of CNS involvement in pediatric CNS metastasis. Intervals to CNS involvement differ between tumor types, suggesting a need for different monitoring strategies.

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Hypervascular filum terminale in Ehlers-Danlos Syndrome patient with tethered cord: A case report and retrospective institutional review

L Cross[1], P Klinge[2], M Punsoni[2]
[1]Brown University Department of Pathology / Lifespan Health System; [2]Brown University / Lifespan Health System

Background: A 39-year-old-female with Ehlers-Danlos Syndrome (EDS) presents for surgical evaluation with multi-year history of progressive back and bilateral leg pain, parasthesias, gait abnormality, headaches, postural orthostatic tachycardia syndrome (POTS), and urinary hesitancy. Physical exam was notable for bilateral clonus and poor proprioception in the ankles/feet. Urodynamic studies showed evidence of a neurogenic bladder. Additionally, no evidence of thickened or low-lying conus was present on MRI images of the entire spine. The diagnosis of occult tethered cord was therefore established in the absence of abnormal MRI findings. A lumbar laminectomy for microsurgical resection of filum terminale was performed. Intraoperatively, the filum was hypervascular with a markedly enlarged and dilated vein. The histopathology of the filum revealed fibroconnective tissue with numerous dilated and congested blood vessels, a well-defined ependymal cell-lined canal, and embedded and adjacent peripheral nerves and ganglion cells. Within 3 months post-surgery the patient reported improved activity levels, with reduction in leg pains, improved gait, and mild improvement in urinary and POTS symptoms.
Methods: Retrospective analysis of our institutional pathology reports for all fila terminalia resected in patients undergoing surgery for tethered cord syndrome from January 2017 to January 2023 identified 74 out of 631 specimens to have enlarged, congested, or otherwise prominent blood vessels. Clinical histories for 51 patients were reviewed to assess symptoms and post-surgical improvement.
Results: At 3 to 6 months post-surgery, 36 patients reported significant improvement in symptoms (23/36 patients with EDS), while 15 patients described little to no improvement (4/15 patients with EDS). Chi-squared analysis comparing EDS status and likelihood of positive surgical response resulted in a p value = 0.015.
Conclusions: Hypervascularity of fila terminalia in our series correlates with greater improvement after surgery, which suggests the potentially important role of dynamic blood flow and increased vascularity in tethered cord syndrome in EDS patients.

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Survey of neoplastic and malformative lesions in patients with surgical resection for epilepsy at a single institution

V Smith, S Wang
Duke University Health System

Background: Epilepsy and otherwise intractable seizures that can be localized to a specific brain location have long been treated with surgical resection when medical treatments are exhausted. Lesions associated with epilepsy include brain neoplasms and developmental malformations. Previously established glial/glioneuronal neoplasms that have been

identified as being responsible for seizure activity includes dysembryoplastic neuroepithelial tumor (DNET), ganglioglioma, gangliocytoma, low grade gliomas, polymorphous low grade neuroepithelial tumor of the young (PLNTY), and others. Among developmental malformations, cortical perivascular satellitosis (CPS) is a rare cortical malformation with only two cases described in the literature. We also previously identified a probable PLNTY with associated CPS. While molecular analysis of neoplastic tissue has become an integrated part of diagnosing neuropathologic lesions, histologic assessment remains central to diagnosis of neoplasms and malformations that lead to seizure activity in the brain. We sought to discern the spectrum of neoplastic and malformative lesions in patients undergoing surgical resection for epilepsy at our institution.

Methods: Therefore, we retrospectively reviewed cases from 81 patients treated for intractable seizures or epilepsy at Duke with a surgical resection per case requisition information between 10/1/2014 and 12/31/2018. We sought to identify cortical perivascular satellitosis and low grade glioneuronal neoplasms within this population. Therefore, oligodendrogliomas, which can histologically mimic PLNTY, and high grade gliomas were excluded from review.

Results: We found that among patients treated for intractable seizures or epilepsy at Duke with a surgical resection, 7% had an associated CNS neoplasm and 2% had CPS.

Conclusions: Thus, we find that in our population CPS is indeed quite rare. A detailed analysis of the range of pathologic findings is still underway, but overall, we find that the spectrum of malformative and neoplastic lesions found in patients at Duke is reflective of the current literature.

SATURDAY POSTERS: Infectious

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CSF immune factor profiling in support of CNS disease diagnosis is improved by the inclusion of complement factor levels

M Curtis[1], C Chen[2], R Kean[3], A Cárdenas[4], M Pettengill[5], J Nassur[6], D Corao-Uribe[7], A Walter[7], A Viaene[4], D Hooper[3], M Curtis[3]
[1]Department of Pathology; [2]SKMC of Thomas Jefferson University; [3]Thomas Jefferson University; [4]Children's Hospital of Philadelphia; [5]Thomas Jefferson University Hospital; [6]SKMC at Thomas Jefferson University; [7]Nemours Children's Hospital Delaware

Background: The human complement system includes body fluid and membrane proteins that play roles in both innate and adaptive immunity. Complement factor modulation of CNS immune environments occurs in response to invading pathogens, neoplasm and autoimmune disorders. Complement activation can be beneficial or harmful. Beneficial complement factor activities include but are not limited to fighting bacterial infections. Harmful complement activation is more likely associated with viral infections or autoimmune disorders. When driven by neoplasms, complement activation can be varied and promote either anti-tumor outcomes or foster pro-tumor microenvironments. These observations suggest that the levels of different complement factors in CSF reflect disease states and may have diagnostic utility.

Methods: To test this hypothesis we measured levels of C1q, C2, C3, C3b/iC3b, C4, C4b, C5, C5a, B, H, Factor D adipsin, and mannan-binding lectin in CSF samples from 26 patients with no CNS disease and from patients with CNS neoplasms (12), bacterial infections (18), viral infections (19), and autoimmunity (6).

Results: Complement factor 2 (C2) showed significantly higher CSF levels in CNS bacterial infections compared to controls ($P < 0.0001$), brain tumors ($P = 0.0254$) and viral infections ($P = 0.0002$) using Mann-Whitney non-parametric test. The area under the curve (AUC) of the receiver operating characteristic curve (ROC) is considered a value for diagnostic accuracy of a "test". The AUC for C2 in bacteria versus control was 0.9496, an "outstanding" range level. C2 bacteria versus viral AUC was 0.8338, an "excellent" value. C5a CSF levels also showed significant statistical differences and "excellent" AUC values between autoimmune and viral infections and between bacterial and viral infections. C3b/iC3b levels between autoimmune and brain tumor specimens were significantly different and displayed an "adequate" AUC value.

Conclusions: These findings support the concept that analysis of CSF complement factor levels can help define CNS disease type-specific immune environments, with implications for both diagnosis and therapy.

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Nocardia brain abscesses: A clinicopathological study of 7 cases

J Lu, V Bass, M Kisiel, C Fong, A Graham, M Lannon, K Reddy, J Lu
McMaster University

Background: Nocardia brain abscesses are rare bacterial infections of the brain, with or without leptomeningeal involvement, usually seen in immunocompromised patients and associated with a high mortality rate. Clinical manifestations of brain Nocardiosis are commonly insidious and nonspecific with symptoms including alterations in consciousness, seizures, headache, nausea, and vomiting. Imaging of brain Nocardia infection is also nonspecific, although it may present with more multifocal lesions, concentric rims, or a multiloculated appearance compared to other pyogenic abscesses or neoplasms. Pathological examination of brain Nocardiosis is essential for diagnosis and appropriate treatment.

Methods: Here, we present a series of 7 patients (ages: 61 - 74 years, with the mean of 68 years; 5 male and 2 female) with pathologically confirmed Nocardia brain abscesses over the past 20 years.

Results: Patients were immunocompromised (4/7) or immunocompetent (3/7), and presented with generalized neurological symptoms such as cognitive changes (6/7), head-

ache (5/7), and nausea (4/7)/vomiting (3/7); as well as focal neurological deficits in concordance with lesion location. Only one patient reported systemic infectious symptoms. On imaging, five abscesses were unifocal while the remaining two were multifocal. All cases, however, were multiloculated with necrotic foci on histopathological examination. Lesions were located in cerebral lobes (5/7) and/or cerebellum (3/7, including one multifocal case). In four cases the lesions appeared tumor-like such that a neoplasm was the top differential diagnosis prior to histopathological examination. These abscesses were histologically in different stages (I-IV), with 5 brains containing stage IV (late encapsulation) foci. Four cases of brain abscesses involved the leptomeninges, and three cases showed the lesions containing plasma cell foci along with macrophage/microglial and lymphocytic infiltrates.
Conclusions: Our findings further characterize the clinical, radiological, and pathological features of Nocardia brain abscesses. As such, this series increases awareness of brain Nocardiosis to clinical and radiological preoperative/differential diagnoses for unusual or complex lesions.

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Chronic Granulomatous Herpes Encephalitis in a Child Surviving Neonatal Disseminated Herpes Infection

A Bakht[1], W Harrison[2], P Lantz[2]
[1]Wake Forest University School of Medicine; [2]Wake Forest University School of Medicine

Background: Herpes simplex virus (HSV) encephalitis is one of the most devastating forms of encephalitis and remains the most common form of sporadic viral encephalitis in the western world. Around 90% of HSV encephalitis cases in adults and children are caused by HSV-1, but in neonates and immunocompromised patients the cause is usually HSV-2. Most cases of acute herpes encephalitis are monophasic; however, in approximately 25% of the cases, relapse has been reported weeks, months, or years following initial infection. In extremely rare instances, HSV encephalitis can be a long-standing central nervous system disorder.
Methods: We present the case of a 2-year-old female who died unexpectedly. When she was a neonate, she developed disseminated herpes infection with encephalitis after contracting HSV-2 from her mother who had active vulvar lesions during delivery. While she survived, she was in a permanent severely devastated neurological state and required complete and total support from caregivers.
Results: An autopsy showed global cystic encephalomalacia of the cerebral hemispheres. The diencephalon, brainstem, and cerebellum were relatively preserved and histologic examination of these structures showed florid encephalitis with frequent giant cells, microglial nodules, and granulomas. IHC highlighted scattered neurons infected with HSV.
Conclusions: This case highlights a rare and poorly understood complication of HSV encephalitis, which neuropathologists need to keep on the differential in deaths of those who survive acute herpes encephalitis as a child.

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Non-granulomatous meningoencephalitis with Balamuthia mandrillaris mimicking a tumor

A Gilani[1], S Enam[2]
[1]University of Colorado, Aurora Colorado;The Aga Khan University, Karachi, Pakistan; [2]The Aga Khan University

Background: Intracranial infections with free-living amoebae are rare but can mimic a neoplastic process on imaging. Naegleria fowleri causes fulminant rapidly fatal acute meningoencephalitis. Acanthamoeba and Balamuthia species on the other hand have a less fulminant presentation but are equally lethal. Here we present a case of sub-acute encephalitis caused by Balamuthia mandrillaris. A 33-year-old woman presented with history of vomiting for 2 weeks, headache for a month and back-pain for 2 months. History was negative for fever, recent travel, history of aquatic activities or recent exposures to animals. MRI of brain showed a large heterogenous enhancing mass involving right temporal and frontal lobes with surrounding vasogenic edema and midline shift.
Methods: Review of clinical and radiologic findings. Routine histochemistry and custom PCR and Sanger sequencing.
Results: Histopathology showed marked lymphoplasmacytic and histiocytic leptomeningitis and encephalitis. Several vessels showed transmural inflammation and damage with perivascular arrangement of amoebic trophozoites. The trophozoites had pale vesicular nuclei, prominent nucleoli, foamy voluminous cytoplasm with PAS+ granules. Rare RBC engulfment was noted. There were no definitive cysts and although there were rare multinucleate giant cells, a prominent granulomatous reaction was not present. The final histopathologic diagnosis was Amoebic meningoencephalitis. The larger size of putative organism favored Balamuthia or Acanthamoeba as the culprit. PCR as well as Sanger sequencing was positive for Balamuthia mandrillaris. Despite comprehensive antibiotic treatment, she continued to worsen, leading to her demise within 2 weeks of presentation.
Conclusions: This is the first confirmed case of Balamuthia mandrillaris CNS infection from Pakistan. The incidence of this disease is expected to rise due to climate change and deteriorating quality of water supply. Balamuthia meningoencephalitis should therefore be on the differential for non-neoplastic CNS lesions. Furthermore, atypical histopathologic picture including absence of granulomatous inflammation needs to be recognized.

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Mycobacterium bovis infection of the brain following intravesical BCG treatment

S Schwartz, S Amaya, J Kirby, H Varma
Beth Israel Deaconess Medical Center

Background: Bacille-Calmette-Guerin (BCG) has been utilized for intravesical treatment of high-risk stage 0a bladder cancers since the 1980s. Intravesical BCG therapy contains the live-attenuated form of Mycobacterium bovis, and is an effective treatment of early-stage bladder cancers. Documented adverse reactions are infrequent, with central nervous system (CNS) complications being exceedingly rare. Here, we report the case of an 88-year-old man who was diagnosed with papillary urothelial carcinoma, then developed non-responsiveness and slurred speech once on BCG therapy.
Methods: MRI revealed a 3.1 cm dural-based left frontoparietal mass on initial presentation. The patient underwent a craniotomy and resection of the mass. Two additional resections were performed following MRI detection of new lesions.
Results: Neuropathological examination of the resected brain tissue revealed granulation tissue formation, necrosis, and lymphohistiocytic inflammation. Gram, GMS, and AFB stains performed on the resected tissue did not reveal any organisms and cultures were negative. Universal PCR was similarly negative for bacteria, fungi, and non-tuberculous mycobacteria. A mycobacterial culture from abscess fluid from the final resection resulted as positive for M. tuberculosis complex, after the patient had been discharged to home hospice care and nine days before his death. The sample was sent to the Centers for Disease Control and Prevention, where further testing revealed the organism to be M. bovis.
Conclusions: This case represents an extremely rare complication of intravesical BCG therapy. The detection of M. bovis organisms in abscess fluid from the brain suggests a likely hematogenous spread from the bladder to the CNS. BCG infections should be considered in the differential diagnosis of unusual abscesses and CNS infections in patients with a history of BCG treatment so that appropriate therapy can be instituted early in the disease process.

SATURDAY POSTERS: Other Topics

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Spatial Transcriptomics on En Bloc Hippocampal Resections from Adult-Onset Drug Resistant Mesial Temporal Lobe Epilepsy Hippocampal Sclerosis

S Guzman[1], S Edminster[2], S Hazany[3], N Shankar[4]
[1]University of Colorado Anschutz Medical Center; [2]University of Southern California LAC-USC; [3]University of Southern California Keck Medical Center; [4]University of Colorado

Background: Adult-onset drug-resistant Mesial Temporal Lobe Epilepsy (MTLE) with Hippocampal Sclerosis (HS) has a poorly understood pathophysiology. In 2013, the International League Against Epilepsy (ILAE) classified the neuropathological patterns of hippocampal sclerosis into 4 types in cases of MTLE. Patients with HS ILAE type 1 have a much longer seizure free period compared to other classifications of hippocampal sclerosis. The reason why this happens is unclear and needs to be further analyzed.
Methods: Our project aims to compare the genetic expression of en bloc hippocampal resection of patients with MTLE HS ILAE type 1 (classical HS) to age-matched autopsy controls without Central Nervous System disease. We utilized Spatial Transcriptomics (ST), 10X Genomics Visium, which is a technique comparable to single-cell RNA-sequencing (RNA-seq), with the added advantage of spatial information. As a result, the dysregulated Differentially Expressed Genes (DEGs) can be assessed in their barcoded spatial context on the hippocampus. Ammon's horn (cornu ammonis) normally separates cells into 8-9 clusters, based on t-distributed stochastic neighbor embedding (t-SNE) and uniform manifold approximation and projection (UMAP) characterization.
Results: Case: 53-year-old female with drug resistant left MTLE with HS and pathologic diagnosis of HS ILAE type 1, classical HS. ST Highlights The dentate gyrus had many DEGs responsible for regulation of growth and proliferation of cells (alpha-1-AR), genes that regulate the number of excitatory synapses (C1QL2), genes that modulate axonal architecture (SNCG), and genes involved in cell morphogenesis and cell motility (LRATD1). The CA3/CA2 region revealed DEGs significant for neuron growth arrest (GADD45G) and transcriptional repressors (AEBP1), as well as other signaling transcriptional regulators. The CA4 neurons revealed many more neurotransmitter and hormone receptors like glutamate receptor binding (SHISA6), insulin-like growth factor I binding (IGGBP5) and strong acetylcholinesterase (ACHE).
Conclusions: While our findings are interesting, we hope to cover the underlying mechanism of HS ILAE type 1.

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Diagnostic yield of post-mortem brain examination following pre-mortem brain biopsy for neoplastic and non-neoplastic disease

C MacRae[1], K Grieco[2], I Solomon[3]
[1]Brigham and Women's Hospital & Harvard Medical School; [2]Brigham and Women's Hospital; [3]Brigham and Women's Hospital & Harvard Medical School

Background: Medical autopsies have decreased over the last several decades, due in part to advances in radiological techniques and increased availability of molecular and other ancillary testing. Pre-mortem diagnosis of central nervous system (CNS) disease with biopsy specimens remains challenging; while ~90% of brain tumor biopsies are diagnostic, 20-70% of biopsies for presumed non-neoplastic or unclear etiologies result in a specific diagnosis. The added benefit of performing post-mortem brain examination on patients who have undergone pre-mortem brain biopsies is not well defined.
Methods: We performed a retrospective analysis of patients who underwent a pre-mortem CNS biopsy and post-mortem neuropathological evaluation at a large academic medical center in Boston, Massachusetts between 2005 and 2022.

Results: A total of 133 cases (37% female; median age 57 [IQR 49-67] years) were identified, including 97 (73%) patients with primary CNS neoplasms, 13 (10%) with metastatic tumors, and 23 (17%) with non-neoplastic neurological disease. Diagnostic concordance between pre-mortem biopsy and post-mortem diagnosis was excellent both for primary CNS neoplasms (98%) and metastatic tumors (100%). Many of these patients were enrolled in clinical trials, and autopsy facilitated the collection of post-treatment tissue to further support brain tumor research. Conversely, patients with non-neoplastic disease only received definitive pre-mortem diagnoses in 3/23 (13%) cases, including progressive multifocal leukoencephalopathy, cerebral mucormycosis, and cerebral amyloid angiopathy. Five (22%) additional patients received conclusive diagnoses following post-mortem examination, including radiation injury/necrosis, CMV encephalitis, Powassan encephalitis (n = 2), and primary leptomeningeal gliomatosis. In the remaining 15 (65%) cases, a more specific differential diagnosis was reported following post-mortem examination compared to the prior biopsy.
Conclusions: These findings highlight the diagnostic challenges of non-neoplastic conditions of the CNS, as well as the diagnostic and research value in performing post-mortem brain examination in patients with both neoplastic and non-neoplastic disease.

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Folic Acid Deficiency-associated Spongiform Leukoencephalopathy in an Autopsy Case of Late-onset SLE with Thrombotic Microangiopathy

H Miyata[1], K Hasue[2], S Hongo[3], A Saitoh[4], T Hatakeyama[4], T Tokairin[4]
[1]Department of Neuropathology, Research Institute for Brain and Blood Vessels, Akita Cerebrospinal and Cardiovascular Center; [2]Akita University School of Medicine; [3]Postgraduate Clinical Education Center, Japanese Red Cross Akita Hospital; [4]Japanese Red Cross Akita Hospital

Background: Cobalamin and folic acid are necessary for the maintenance of the myelin sheath. Deficiencies of these vitamins can cause vacuolar myelopathy, and cerebral white matter lesions have rarely been reported in patients with folate deficiency.
Methods: A 78-year-old woman presented with a fever and was hospitalized under suspected pyelonephritis. Subsequent laboratory examinations revealed pancytopenia with macrocytic anemia, hypocellular bone marrow, and low serum folic acid concentration (0.5ng/mL; reference value, 3.6-12.9 ng/mL) (day 45), which improved to 7.6 ng/mL a month later or ten days before death after the treatment with granulocyte colony-stimulating factor and folic acid supplementation. Serum cobalamin concentration was over 1,500 pg/mL due to intravenous fluid administration. Other laboratory data included positive serum anti-nuclear and double-strand DNA antibodies and proteinuria, and the clinical diagnosis of systemic lupus erythematosus (SLE) was made on day 65. On day 71 or 14 days before death, she presented with thrombocytopenia (3,000/μL) and was treated with steroid pulse therapy for three days, which was ineffective. SLE-associated thrombotic microangiopathy (TMA) was suspected based on the presence of fragmented red blood cells in the peripheral blood. The patient died of a sudden cardiac arrest on day 85.
Results: Neuropathological examination of the fixed brain revealed scattered microscopic infarcts of varying stages in the cerebral and cerebellar cortices. Frequent hyaline eosinophilic platelet microthrombi with occasional mural fibrin was observed in the brain parenchymal microvessels, and endothelial hyperplasia and necrotic microvessels without an inflammatory reaction were scattered in the cerebral cortex, supporting the clinical diagnosis of TMA. A widespread diffuse vacuolar change or spongy state was prominent in the white matter, characterized by myelin sheath ballooning with preserved axons without significant astrocytic and microglial reactions.
Conclusions: These pathological findings, with biochemical considerations, suggest a central role for folate deficiency in the pathogenesis of TMA and spongiform leukoencephalopathy.

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2022 Annual Report of the Japanese Brain Bank Network for Neuroscience Research

S Murayama[1], Y Saito[2], D Kaneda[3], B Mihara[4], M Takao[5]
[1]United Graduate School of Child Development, Osaka University; [2]Tokyo Metropolitan Institute for Geratrics and Gerontology; [3]Fukushimura Hospital; [4]Mihara Memorial Hospital; [5]National Center of Neurology and Psychiatry

Background: The Japanese Brain Bank Network for Neuroscience Research (JBBNNR), established in 2016, welcomed the refunding of Grant-in-Aid for Transformative Research Areas, MEXT, Japan from 2022 to 2027, whose center is the Brain Bank for Aging Research (BBAR), the first and only Japanese Brain Bank dedicated for aging and dementia research.
Methods: JBBNNR, in close collaboration with the Brain Bank Committee, the Japanese Society of Neuropathology (JSNP), recruits brain donors, propagates the BBAR protocols for neuropathological examination, and keeps the policy of open resource. JBBNR member consists of BBAR, the Brain Bank for Neurodevelopmental, Neurological and Psychiatric Disorders (BBNNPD) of Osaka University, National Center of Neurology and Psychiatry, Mihara Memorial Hospital and Fukushimura Hospital.
Results: In 2022, the effect of the pandemic of COVID-19 influenced the autopsy recovery of postmortem brains, owing to the recommendation of the National Institute of Infectious Disease (NIID), Japan, not to use electrical scissors in autopsy. We conducted a small number of COVID-19 autopsies with prion format, following the informal permit of NIID. The effort to include legal autopsy of suicide victims and accidental death cases of developmental disorders in BBNNPD experienced difficulty in Japanese culture and law. JSNP acknowledged ten board certified neuropathologists, one of whose aims is to keep the Japanese brain bank network. JBBNNR is based on full au-

topsy and could support brain- first, body- first hypothesis of Lewy body disease (LBD), demonstrating the initial deposition starting either from olfactory bulb or sympathetic ganglia. The high- quality resource of JBBNNR contributed to identification of Lewy fold with cryo- EM by a Cambridge group published in Nature.

Conclusions: JBBNNR continues to contribute to neuroscience research, focused on Alzheimer disease and LBD in Japan. (We will dedicate this paper to late Ms. Sayoko Komiyama who continuously supported our activity and died of COVID 19.)

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A Systematic Review and Meta-analysis of Brain Weight at Autopsy: A critical examination of fundamental assumptions

M Chambers, F El-Ghazali, C Keene, A Nolan
University of Washington

Background: 1200-1500 g is a common convention for adult brain weight. However, large autopsy surveys are predominantly composed of old data without standardized methodology nor adequate exclusion criteria. Herein we undertake a systematic review and meta-analysis of normal brain weight at autopsy.

Methods: A systematic search identified studies which recorded brain weight at the time of autopsy among the general population. Exclusion criteria included < 100 brains, inadequate data, and studies which were restricted to specific diseases. Included studies were aggregated for pooled estimates of average weight and standard deviation (SD). Analysis of heterogeneity and risk of bias were assessed across studies.

Results: 22 articles published between 1880 and 2022 met inclusion criteria. Among 33,021 brains (21,166 male, 11,855 female), the average brain weight was 1,324 g (SD: 108 g). Most studies were low quality (n = 11) being composed of hospital patients with inadequate exclusion criteria. Seven studies (n = 7,038) were high quality, predominantly composed of forensic cases with clear exclusion criteria. Brain weights in low quality studies were significantly smaller compared to high quality studies (p = < 0.001). Among high quality studies, the average brain weight for males was 1,422 g (n = 4,911, SD: 129 g) and for females 1,274 g (n = 2,127, SD: 110). Data on brain weight by sex and age confirmed a linear decrease in brain size in males and females with increasing age. There was an average loss of approximately 100 g in women and men (95 g and 100 g respectively) between the third decade and the eighth decade.

Conclusions: The results suggest that the most cited articles for standard brain weight at autopsy are of poor quality. Additionally, 1,200-1,500 g for normal adult brains normalizes heavy female brains and small male brains. More accurate representations of adult brain weight consider sex and age.

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Time is of the Essence: Examining How Post-Mortem Interval Impacts Brain Cell Morphology and Mechanisms of Decomposition

M Krassner[1], J Kauffman[1], S Walsh[2], K Farrell[1], A McKenzie[1], J Crary[1]
[1]Icahn School of Medicine at Mount Sinai; [2]CUNY Hunter College

When assessing autopsy brains in research and clinical contexts, the post-mortem interval (PMI) has a significant impact on multiple factors, including the integrity of macromolecules, but the changes to brain cell morphology are less understood. We screened 2119 abstracts, 361 manuscripts, and selected 172 studies to investigate the effect of PMI on brain cell structure using a qualitative meta-analysis. For each publication, cellular degradation outcomes were extracted and rated by multiple reviewers on a 0-3 scale (absent/minimal, partial, severe, or near-total decomposition). We then examined how cellular features, such as membrane quality, are influenced by PMI. Mechanistically, fluid accumulation causes volume changes at earlier time points, while complete disintegration of the cell membrane occurs later. Other common decomposition mechanisms include oncotic cell death, subcellular vacuolization, and biomolecule redistribution and degradation. Conditions such as storage temperature and premortem pathology further influence post-mortem decomposition rate. Certain visualization methods (e.g., immunohistochemistry) are especially sensitive to PMI because some antigens degrade rapidly. The intraclass correlation (ICC), a measure of inter-rater reliability, was 0.72 (moderate reliability, p = 7.9 x 10-43), reflecting limitations in both the clarity of the categories we defined and the precision by which the observations were described. There is a wide range of PMIs after which histology reaches different decomposition severities across studies, experimental designs, and structural features. We identified a significant positive rank correlation between the PMI and decomposition severity score when pooling observations ($\rho = 0.29$, p = 4.0 x 10-7). We conclude that there may be advantages to examining some cellular features in biopsies, rapid autopsies, or in vivo animal models as they are influenced by the PMI. More investigation will further clarify the impact of PMI on brain cell structure.

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Thiamine Deficiency Due to Cessation of Food Intake: Wernicke Encephalopathy and Beriberi as Possible Outcomes

F Davis[1], M Maher[1], E Williams[2], P Buccini[2], P Boyer[1]
[1]ECU Health Medical Center; [2]Brody School of Medicine, East Carolina University

Background: Deficiency of thiamine, an essential, water-soluble vitamin, is rare in developed countries and most often associated with malnutrition, severe oral intake restriction, and

bariatric and gastric bypass surgeries. Neurologic manifestations of thiamine deficiency include cognitive impairment with Wernicke encephalopathy and peripheral neuropathy without or with heart failure, dry and wet beriberi, respectively. This report summarizes two cases of severe thiamine deficiency due to severely reduced food intake with distinct clinical manifestations.

Methods: The patients' electronic medical record was reviewed and a literature review using appropriate key words was performed.

Results: Case 1: A 36-year-old, incarcerated man with a history of schizophrenia undertook a month-long hunger strike with estimated weight loss of 50 pounds. He presented with altered mental status. Magnetic resonance imaging identified T2-bright signal around the medial thalamus, fornix, mammillary bodies, and the periaqueductal and peri-fourth ventricular regions. Laboratory evaluation identified a thiamine level of < 6 nmol/L (reference range: 70-180 nmol/L). He suffered a myocardial arrest. Post-mortem neuropathologic evaluation identified marked vascular congestion, hemorrhage, and macrophage infiltrates in the regions of imaging abnormality consistent with acute to early subacute Wernicke encephalopathy. Case 2: A 35-year-old woman who underwent a 20-day "water fast" with no food intake and a self-reported 65 pound weight loss presented with lower extremity weakness and pain. Electrodiagnostic evaluation revealed severe acute sensorimotor axonopathy. An extensive peripheral neuropathy laboratory workup revealed only thiamine deficiency, 27 nmol/L. The diagnosis of dry beriberi was made and, following a month of vitamin supplementation and physical therapy, she showed improvement in mobility.

Conclusions: These cases underscore the dangers of non-medically supervised, severe reduction in food intake. With limited storage and a 14-30 day reserve, symptoms of thiamine deficiency can manifest within weeks of food restriction. Replacement can reverse some symptoms, but early recognition and intervention is essential to prevent long-term neurological damage.

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Understanding the dysregulation of lipid metabolism in the adult dentate gyrus during seizure

A Ahamad[1], S Ge[2]
[1]Stony Brook University and NYU Langone Health; [2]Stony Brook University

Background: Dentate gyrus (DG), a neurogenic niche, is a metabolically dense subregion of the hippocampus. Continuous production and integration of new neurons in the existing circuit, accompanied by neuron-glia coupling, is essential to maintain hippocampal homeostasis throughout adulthood. Cell metabolism regulates the homeostatic balance of the hippocampal network and its associated functions, such as memory and cognition. An imbalanced circuit activity results in mesial temporal lobe epilepsy (MTLE), impairing the overall network function. Although altered lipid metabolism is reported in status epilepticus, the role of lipid droplets (LDs), metabolically active organelle known to provide a substrate for cellular energy, has not been explored in DG during seizure. LDs are composed of neutral lipids and surrounded by a phospholipid monolayer studded with a structural Perilipin family of proteins 1-5 (PLIN 1-5), reported to be involved in lipid metabolism. However, the underlying role of LD metabolism remains unexplored during seizure.

Methods: In this study, we used a novel approach and labeled LDs in DG by injecting a lipid dye in the tail vein of pilocarpine-induced seizure mice. We sacrificed mice at three-time points, 0.5, 1-, and 3 hours post-seizure induction.

Results: We found a significant increase in LDs accumulation at three-time points post-seizure as compared to control. To elucidate the role of neuron-glia metabolic coupling in DG, we found increased levels of LDs in seizure mice than in the control cohort suggesting seizure dysregulates lipid droplets metabolism in neurons and glia in DG.

Conclusions: Overall, this novel study will highlight the undiscovered role of LDs in the DG during seizure and, in the future, can be used as a therapeutic target to alleviate the MTLE phenotype.

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First reported autopsy brain findings for Cerebellar Hippocampal And basal Nuclei Transient Edema with Restricted diffusion (CHANTER) syndrome

L Ramachandran Nair[1], K Schwetye[2]
[1]Washington University in St Louis; [2]Washington University in St. Louis

Background: Cerebellar Hippocampal And basal Nuclei Transient Edema with Restricted diffusion (CHANTER) syndrome is a recently described clinic-radiologic syndrome. Patients present with decreased level of consciousness with subsequent development of obstructive hydrocephalus. In the two published clinical case series, all patients were acutely intoxicated with opiates or similar drugs of abuse. Brain MRI showed abnormal restricted diffusion in bilateral cerebellar hemispheres, hippocampi and basal nuclei in a symmetric fashion. Most patients had favorable clinical outcomes and no brain biopsy or autopsy specimen examinations were reported.

Methods: Here, we present autopsy brain findings in such a patient, which could be the first ever reported histopathological findings of CHANTER, to the best of our knowledge. The deceased was a 45-year-old male found unresponsive after an unknown duration of "down time" with depressed respirations and pin-point pupils. Narcan improved his oxygen saturation, but he continued to be unresponsive, intubated and on supportive life measures. Urine drug screen was positive for fentanyl and cannabinoids. Brain MRI showed edema and diffusion restriction in the bilateral cerebella, hippocampi, and basal nuclei. He was treated with ventriculostomy and decompressive craniectomy. Due to subsequent worsening of his clinical condition, the family elected for comfort care. He was declared dead 4 days after the presentation.

Results: Grossly, the brain weighed 1270 grams and showed evidence of strikingly symmetric, dark-brownish discoloration in the bilateral basal ganglia, hippocampi and cerebella. Histopathology showed marked hypoxic/ischemic damage in the aforementioned regions with hemorrhage and necrosis, mostly in a subacute pattern. There were some endothelial prominence and occasional perivascular chronic inflammation.
Conclusions: Though no "surprising" or unique histological features were unraveled by autopsy, this case provides an important histopathological correlate to a heretofore clinico-radiologically defined entity. Given the previously-described favorable outcome for most patients and ongoing opioid crisis, a better pathophysiological understanding of this entity is crucial.

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Cross-site Translation of Digital Image Analyses for Quantitative Ki-67 Assessment to Pituitary Adenomas: An Easy Solution?

G Sexton, L Seigh, T Pearce
University of Pittsburgh Medical Center

Background: Assessment of Ki-67 immunolabeling is an important component of evaluating neuroendocrine tumors (NETs) throughout the body. In pituitary adenomas (PitNETs), assessment of Ki-67 proliferative index in combination with radiological findings and tumor histology can be used to identify tumors with the potential for aggressive behavior. With increasing adoption of whole slide imaging and digital pathology into routine workflows, the potential utility of image analysis algorithms for quantitative assessment of Ki-67 proliferative indices is of interest. Here, we examine whether quantitative Ki-67 image analysis algorithms validated for other tumor types and sites of origin can be easily deployed for analysis of pituitary tumors.
Methods: Digitally imaged pituitary adenoma cases signed out at our institution between 2020 and 2021 (n = 113) were identified, and digitized Ki-67 stained slides were annotated for image analysis. Two quantitative Ki-67 algorithms developed by our institution (Leica Aperio platform), one for gastrointestinal NETs and the other for bone marrow, were applied to the pituitary lesions. Algorithm outputs were compared to the originally reported values for Ki-67 proliferative index, and to a detailed manual re-review.
Results: Both algorithms significantly overestimated the percentage of positive nuclei within the pituitary lesions. Review of the generated annotation overlay images showing positive and negative nuclei revealed poor algorithm performance in identification of negative nuclei stained only by hematoxylin counterstain, with differences between the two algorithms.
Conclusions: Despite the sections being created in the same histology laboratory and digitized on the same whole slide imaging scanners, quantitative Ki-67 algorithms developed for other tumor types translated poorly to pituitary NETs. Our results indicate that algorithms developed for use in tumors at one body site require optimization and validation before being applied to similar neoplasms arising in other anatomic locations.

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Molecular Taxonomy of Schizophrenia

C Rhodes[1], E Bowen[2], E Childers2[2], R Granger[2]
[1]DoD/USU Brain Tissue Repository; [2]Dartmouth College

Background: Almost half a century ago, Fred Plum called schizophrenia "the graveyard of neuropathologists", and in many ways the situation has not appreciably changed. In spite of decades of anatomic, histologic, and molecular studies, little progress has been made elucidating the pathobiology of schizophrenia. A longstanding hypothesis to explain this lack of progress is that schizophrenia is a heterogeneous disease and that meaningful results have been obscured in studies which pool data from biologically different patients.
Methods: This is the re-analysis of data made publicly available by scientists at the Lieber Institute.
Results: We previously reported how in the re-analysis of expression array data from the DLPFC of schizophrenic patients and controls, the statistical significance of differential gene expression for almost all of the differentially expressed genes was driven by about half of the schizophrenic patients. In other words, the schizophrenics patients could be divided them into two groups, "type 1" patients who have a DLPFC transcriptome similar to that of controls with no genes differentially expressed at a statistically significant level, and "type 2" schizophrenics patients with thousands of differentially expressed genes. In the present study we confirm that result and extend it using RNAseq data from the DLPFC and from the caudate.
Conclusions: Based on these results, we suggest that "schizophrenia" is a syndrome, not a disease, and that on the basis of the underlying molecular pathology two distinct diseases which produce this clinical syndrome can be identified. Going forward it is important that studies intended to elucidate the pathobiology of "schizophrenia" as well as clinical trials of novel therapeutic agents take into account the fundamental biologic difference between type 1 and type 2 schizophrenic patients.

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Case Report: PDGFB-related Primary Familial Brain Calcification Presenting as Brain Mass

E Russler-Germain, S Dahiya, N Ghoshal
Washington University in St. Louis School of Medicine

Background: Primary familial brain calcification, also known as Fahr disease or familial idiopathic basal ganglia calcification, is caused by mutations in PDGFB, PDGFRB, SLC20A2, XPR1, MYORG or JAM2 genes resulting in pericyte dysfunction and inorganic phosphorous accumulation. Neuroimaging demonstrates bilateral brain calcifications, commonly in the basal ganglia but also in the cerebellum, thalamus, or brain-

stem. Patients typically present around age 30 with movement disorders, headaches, psychiatric symptoms, or seizures.
Methods: Here, we present the case of a 60 year old man with a history of cerebellar degeneration who presented to the emergency room with worsening left-sided weakness. Head CT showed bilateral basal ganglia and white matter calcifications. Brain MRI showed a large area of edema in the distribution of the right middle cerebral artery involving the majority of the right basal ganglia and adjacent white matter with mass effect, concerning for a neoplasm or large subacute infarct.
Results: Biopsy of the right basal ganglia lesion was performed, showing white matter with marked reactive changes including gliosis, edema, microinfarction, and perivascular and parenchymal chronic inflammatory cell infiltration. Extensive microcalcifications in different shapes were present throughout the specimen, some deposited in and around the blood vessels and some in the brain parenchyma itself. These calcifications were surrounded by robust tissue reaction including foreign-body type giant cells and chronic inflammatory cells. Blood vessel walls were destroyed by calcification and accompanying tissue inflammation to varying degrees, including a few foci of complete luminal occlusion. In conjunction with the presence of bilateral basal ganglia calcifications, the biopsy raised the possibility of a familial etiology of disease. The patient underwent whole exome sequence analysis and was found to have a PDGFB pathogenic variant.
Conclusions: This case represents an unusual clinical presentation of PDGFB-related primary familial brain calcification, with mass-like lesion caused by calcification-induced vasculitis, vasculopathy, and associated infarction.

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Ultrafast molecular characterization of brain tumors

C Lin[1], F Emiliani[2], A Ould Ismail[1]
[1]Dartmouth-Hitchcock Medical Center; [2]Dartmouth College

Background: Glioblastoma is the predominant type of primary malignant brain tumor. Although there have been significant advancements in surgical procedures and chemoradiation therapies, the overall prognosis remains dismal, with a median survival rate of 15-18 months. In nearly all cases, glioblastomas will relapse locally during the course of treatment. Applying molecular targeted drugs during surgery may provide superior therapeutic results, prevent local recurrence, and minimize systemic side effects. However, current sequencing methods (such as next-generation sequencing and array-based hybridization) are laborious and time-consuming, prohibiting such applications.
Methods: Here, we develop an innovative enzyme-based method to randomly reconstruct genomic DNA for analysis with 3rd generation sequencing technologies (Oxford Nanopore Technologies). By exploiting the irreversible ligation of compatible sticky ends, we are able to perform simultaneous reactions using restriction endonuclease and T4 DNA ligase to achieve DNA reconstruction. We have successfully applied this technique to investigate copy number variations (CNVs) in both normal and diseased brain tissue.
Results: Our preliminary findings demonstrate that the optimal efficiency for genomic DNA reconstruction is achieved at 30 minutes. When coupled with one hour of real-time sequencing, we achieve a genomic resolution of 3.3 kb for copy number variation analysis. This is superior to all current genome-wide CNV assays, including Oncoscan and comparative genomic hybridization. Moreover, the entire process could be accomplished within 120 minutes upon receipt of fresh tissue.
Conclusions: Our molecular diagnosis technique has the potential to become the gold standard for CNV analysis and may pave the way for molecular targeted therapy during surgery. We believe that our approach will significantly improve patient outcomes and ultimately become a routine part of patient care.

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Intraoperative Confocal Laser Endomicroscopy: Differential distribution of the staining dye between different tumor types

J Schlegel[1], M Brielmaier[1], J Reifenrath[1], N Pensel[1], A Wagner[2], J Gempt[3], J Schlegel[1]
[1]Technical University Munich; [2]Klinikum rechts der Isar, Technial University Munich; [3]University of Hamburg Medical Center Eppendorf

Background: Confocal Laser Endomicroscopy (CLE) offers in vivo imaging of living tissues. The distribution of the staining agent sodium-fluorescein (NaFl) plays a key role in establishing in vivo CLE as a new opportunity for intra-operative real-time imaging. Sodium-fluorescein is used as a fluorescent tracer in neurosurgery. By comparing the distribution of NaFl in CLE with conventional fluorescence microscopy of NaFl incubated tumor cells we might gain a better understanding of the staining kinetics.
Methods: Here, we applied the staining agent intravenously at the beginning of the surgical procedure. In vivo CLE of the lesion was performed 30 to 50 minutes later using the Zeiss CONVIVO confocal microscope (Zeiss, Oberkochen, Germany), and compared with conventional fluorescence microscopy. In addition, different tumor cell lines derived from malignant gliomas and carcinomas, respectively, were incubated with NaFl in vitro and the uptake of the fluorescent dye was monitored over time.
Results: The intraoperative images showed specific fluorescein distribution depending on the architecture of the tumor type. Gliomas demonstrated accumulation of the staining agent in the extracellular tumor matrix, whereas cells of metastases from carcinomas appeared to take up more sodium-fluorescein intracellularly. These results were corroborated by NaFl uptake in cell culture experiments. In vivo CLE imaging offered a fast assessment of the tissue allowing intra-operative tumor diagnoses. The specific distribution of the fluorescent agent NaFl helped for a discrimination between the different neoplastic entities. Images from in vivo confocal laser

endomicroscopy showed NaFl uptake in concordance with the results of NaFl incubated cell cultures.
Conclusions: Intraoperative, in vivo confocal laser endomicroscopy shows promising first results in the understanding of brain tumor histomorphology in situ. Being faster and less manipulated by artifacts than conventional techniques, it offers wide opportunities for research and intraoperative diagnostics.

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Histopathological analysis of ruptured intracranial aneurysms – An insight into etiopathology

D Lal[1], R Wickremasinghe[2], D Attanayake[3]
[1]National Hospital Kandy; [2]National Hospital Kandy, Sri Lanka; [3]National Hospital of Sri Lanka

Background: Intracranial aneurysm is a significant component of neurosurgical practice and its etiopathology still remains unclear. However, there are evidence for tissue degeneration related pathophysiology; of which atherosclerotic and inflammatory processes are gaining more attention with MRI based vessel wall imaging and immunohistochemical staining techniques. The objective of this study was to identify the relative contribution of atherosclerosis and inflammation to pathology of intracranial aneurysms based on patient's age which is a reflection of tissue degeneration.
Methods: 60 patients with ruptured intracranial aneurysms, diagnosed based on CT angiography +/- Digitally subtracted angiography, treated with surgical clipping over a period of one year in a single neurosurgical unit was included in the study. Unruptured aneurysms and patients who were not suitable for surgical treatment were excluded. Aneurysm dome was resected micro surgically after clipping and histopathology was analyzed with routine staining to identify atherosclerosis and inflammation. Patient's gender and anatomical distribution of the aneurysm were not considered for stratification.
Results: Inflammatory degeneration and atherosclerotic degeneration were predominantly seen in 08(66.7%) and 04(33.3%) patients bellow 39 years of age, 13(61.9%) and 08(38.1%) patients in 40 to 59 years of age, 15(55.6%) and 12(44.4%) patients above 60 years of age respectively.
Conclusions: Inflammatory degeneration is the main pathological process in all ages and the risk of atherosclerotic degeneration increases with the age. This could represent the known sequalae of inflammation leading to atherosclerosis in vessel wall with aging and confirm the inflammation, rather than atherosclerosis as the primary etiopathology of intracranial aneurysms.

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Unusual case of intraparenchymal hemorrhage: 32-year-old with hypertension, hypertrophic cardiomyopathy, & severe systemic atherosclerosis

L Evans, D Marshall
University of Washington

Background: A 32-year-old male without known past medical history presented to the emergency department with acute-onset slurred speech, left-sided weakness, and mydriasis post-coitus. He was severely hypertensive, and imaging discovered a large basal ganglia intraparenchymal hemorrhage, in addition to evidence of multiple remote infarcts in the cerebellum and cerebrum. An emergency hemicraniectomy was performed, however the patient died three weeks after presenting.
Methods: At autopsy, we found severe coronary atherosclerosis and cardiomegaly. Histologic evaluation of cardiac tissue revealed evidence of both acute and remote ischemic infarcts, dysplastic blood vessels in the myocardial microcirculation, and congenital hypertrophic cardiomyopathy (HCM).
Results: The brain grossly demonstrated severe cerebral atherosclerosis, a finding that is extremely rare in patients prior to their fourth decade of life. Complete dissection of the Circle of Willis identified extensive macroscopic and microscopic atherosclerosis in the internal carotid artery (ICA), anterior cerebral artery (ACA), middle cerebral artery (MCA), posterior cerebral artery (PCA), basilar artery (BA), and vertebral artery (VA). In addition to the large right basal ganglia bleed, multiple small remote lacunar infarcts in the bilateral frontal and parietal white matter, left basal ganglia, and left cerebellum were identified. Histologically, lacunar and territorial infarcts with macrophage infiltration and reactive capillary proliferation were seen, consistent with early chronic infarcts correlating with his time of presentation. Additionally, more remote infarcts were identified, underlining the chronicity of his disease. While no evidence of vasculitis was found, severe hyperplastic arteriolosclerosis and evidence of chronic hypertensive change with an "onion skin" appearance of vessels were present.
Conclusions: The patient's severe atherosclerosis and arteriolosclerosis are consistent with chronic, uncontrolled hypertension. The severity seen here is extreme for someone his age without clinical hyperlipidemia or evidence of vasculitis. This odd presentation raises the question of another vasculopathy component, which could be genetic given the finding of congenital HCM as well.

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HTRA1-related small vessel disease: Vascular degeneration revealed by 3D pathologic analysis and fibulin5 immunostaining

R Saito[1], T Kato[2], M Uemura[2], Y Saito[3], S Murayama[4], K Tainaka[2], O Onodera[2], A Kakita[2]
[1]Department of Pathology, Brain Research Institute, Niigata University; [2]Niigata University; [3]Tokyo Metropolitan Geriatric Hospital & Institute of Gerontology; [4]Tokyo Metropolitan Geriatric Hospital & Institute of Gerontology and University of Osaka

Background: We have reported a characteristic vascular pathology in HTRA1-related small vessel disease (HTRA1-SVD), caused by biallelic HTRA1 mutations known as CARASIL, where positivity for smooth muscle actin (SMA) is

minimal and 3D-IHC demonstrates selective loss of SMA in main branches of the vascular network, in comparison with CADASIL and sporadic SVD (AANP 2021). In the present study, we focused on accumulation of fibulin5, which is reported to be highly accumulated in cerebral vessels of HTRA1-SVD mice and evaluated the associated pattern of SMA loss.

Methods: We investigated 5 patients with HTRA1-SVD (homozygous 2, heterozygous 3), 3 patients with CADASIL, 20 patients with sporadic SVD, and 5 individuals without any neurological disorders. Immunohistochemistry for fibulin5 was performed on formalin-fixed paraffin-embedded frontal sections. HTRA1 and NOTCH3 mutations were evaluated in the patients with SVDs.

Results: Immunohistochemistry revealed fibulin5-positive deposits in the subendothelium and elastic lamina of arterioles in the patients with both homozygous and heterozygous HTRA1-SVD. Fibulin5 accumulation on the elastic lamina was evident predominantly in larger-diameter (70-700 μm) arterioles in the subarachnoid space, cortex, and white matter. Immunoreactivity was limited to the subendothelium in other patients, or was negative in younger controls.

Conclusions: The distribution of vessels with fibulin5 immunoreactivity is similar to that of vessels with SMA loss demonstrated by 3D analysis. This selective pattern of SMA loss in HTRA1-SVD may be related to selective fibulin5 deposition in larger arterioles.

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Clinical relevance, and prognostic significance of isolated angiitis of the vasa vasorum in temporal artery biopsies

S Pejhan[1], L Barra[2], P Basharat[2], L Allen[3], L Bursztyn[3], A Proulx[3], R Chen[2], M Smith[4], M Hackett[5], R Hammond[2]
[1]Department of Pathology & Lab Medicine, London Health Sciences Centre, Schulich Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada; [2]London Health Sciences Centre, Schulich Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada; [3]Ivey Eye Institute, St. Joseph's Hospital, Schulich Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada; [4]London Health Sciences Centre, Schulich Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada; [5]Schulich Medicine & Dentistry, University of Western Ontario, London, Ontario, Canada

Background: Temporal arteritis (TA) is a well-known clinical-pathological entity. Untreated, many patients will suffer vision loss and/or stroke. Angiitis of vasa vasorum (AVV) and small vessel vasculitis of the periadventitia (SVV), whose significance is uncertain, may also be present in isolation or in combination with TA.

Methods: We investigated the relevance of small vessel inflammation in temporal artery biopsies. Our dataset consists of 72 consecutive temporal artery biopsies (TAB) and matching retrospective clinical data. Scoring of inflammation on TAB was designed to include vasa vasorum and periadventitial small vessels. Specimens were categorized into 3 subgroups: TA, Isolated AVV (IAVV), and Isolated SVV (ISVV). Follow-up was 1 year post-biopsy.

Results: Microscopy revealed TA in 25% of cases, IAVV in 21% and ISVV in 7%, while 47% were negative. All TA cases had accompanying small vessel inflammation (AVV in 78%, SVV in 5%, and AVV+SVV in 17%). Demographics were similar across the groups. Diplopia and jaw claudication were more common in TA than IAVV/ISVV. ESR and C-reactive protein were higher in TA as well. In patients with IAVV/ISVV, 44% had a moderate to high clinical probability of TA at presentation and 28% acquired a diagnosis of TA during follow-up versus 14% with a negative TAB. The interval between initiation of corticosteroids and biopsy was longer in IAVV/ISVV than in TA.

Conclusions: The significance of small vessel inflammation in temporal artery specimens has been debated. In our study, TA and AVV frequently co-exist, reinforcing their association. Patients with IAVV/ISVV had longer corticosteroid exposure, which may have impacted TAB findings. These patients were also twice as likely to receive a clinical diagnosis of TA in follow-up compared to those with no inflammation on TAB. The effects of corticosteroids, and the segmental nature of vascular involvement in TA should be considered in assessing inflammation, and planning treatment.

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Deep Sea Diving Death due to Ruptured Arteriovenous Malformation: Role of Medical Examiner System

F Davis[1], S Taylor[2], C Johnson[2], K Kelly[1], P Boyer[1]
[1]ECU Health Medical Center; [2]Brody School of Medicine, East Carolina University

Background: Neurological symptoms or death in a diver following a dive elicits a broad differential diagnosis including the possibility of decompression illness or manifestation of an underlying disease process. We report the first autopsy case of a fatal ruptured cerebral arteriovenous malformation (AVM) in the setting of a dive.

Methods: A literature review identified no publications describing ruptured vascular malformations in a diver.

Results: A 40-year-old, experienced male diver became unresponsive on ascension from a dive. Resuscitation efforts were unsuccessful. An unrestricted, forensic autopsy revealed acute, diffuse cerebral swelling and an acute right frontal lobe white matter hematoma at the level of the nucleus accumbens in the context of multiple distended blood vessels. Microscopic evaluation demonstrated variably sized arteries, arterialized and partially sclerotic veins, and admixed neuropil in a background of acute hemorrhage. In addition, plump, active-appearing macrophages with moderately coarse hemosiderin aggregates along with macrophages with fine hemosiderin accumulation and active and chronic gliosis were noted adjacent to the abnormal blood vessels, consistent with subacute and chronic reactive

changes. Findings were diagnostic of a vascular malformation, consistent with the diagnosis of AVM, with evidence of previous subacute and chronic leakage.

Conclusions: This report highlights the critical role that forensic pathology plays in the investigation of unexpected deaths, establishing both (1) the cause of death, in this case acute intracerebral hemorrhage in the context of an AVM, and (2) the manner of death, in this case a natural manner due to rupture of an AVM which is a known complication of this developmental abnormality. This case report emphasizes the role of an autopsy in determining the cause of death of a diver and establishes a ruptured AVM as a potential cause of death during or following a dive.

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Fibromuscular dysplasia and ANCA-associated vasculitis presenting on temporal artery biopsy

B Kleinschmidt-DeMasters
University of Colorado Anschutz Medical Campus

Background: Fibromuscular dysplasia (FMD) is a non-atherosclerotic, non-vasculitic arteriopathy of predominantly medium to small-sized vessels, usually affecting renal (60-75% cases) and extracranial carotid and vertebral (20-30%) arteries. Reports detailing histopathological features of FMD when it is seen on biopsy for evaluation of temporal (giant cell) arteritis; TA/GCA) are virtually non-existent. Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitides (AAVs) can rarely be revealed by TA manifestations, leading to a risk of misdiagnosis of GCA and inappropriate treatments.

Methods: Patient #1 is a 59-year-old male with hypertension (HTN) and several prolonged hospitalizations for recurrent multifocal ischemic strokes. Extensive workup included multiple MRIs, lumbar puncture, CTs of chest/abdomen/pelvis and finally temporal artery biopsy. Patient #2 is an 86-year-old female who presented with 2 months of fevers and myalgias with elevated CPK and inflammatory Markers; she underwent temporal artery biopsy for presumed GCA.

Results: Temporal artery biopsy of Patient #1 revealed FMD involving the temporal artery. MRA showed multifocal stenoses most significantly involving the proximal M2 branches and left A4 branch. Vasculitis workup was negative for cANCA, pANCA, rheumatoid factor IgG, and nuclear IgG, Sjogren's -A and -B, Smith, and double stranded DNA IgG antibodies. Temporal artery biopsy of Patient #2 showed no temporal arteritis; instead necrotizing vasculitis of a medium-sized vessel in adjacent adipose tissue was identified. Rheumatological workup showed pANCA and MPO positivity, meeting criteria for ANCA-associated vasculitis (AAV) more than granulomatosis with polyangiitis (GPA). Unlike 80-90% of GPA, this patient did not have renal involvement.

Conclusions: AAV is a known, but rare, mimic of temporal arteritis/GCA, with one study demonstrating 2.5% of patients that fulfilled the American College of Rheumatology 1990 classification criteria for GCA having AAV, making it's recognition critical for pathologists/neuropathologist. In contrast, FMD is seldom in the differential for patient with TA/GCA.

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Evaluation of Neurodegeneration and Cerebrovascular Disease in Rheumatoid Arthritis Patients

R Larsen, E Constantopoulos, C Crowson, C Hulshizer, C Kodishala, R Kumar, R Lennon, E Lovering, E Myasoedova, A Nguyen
Mayo Clinic

Background: Active rheumatoid arthritis (RA) has been associated with an increased risk of both cardiac and peripheral vascular disease. We aimed to compare cerebrovascular changes in RA patients with matched non-RA patients, both with and without a neuropathologic diagnosis of neurodegenerative disease.

Methods: RA ($n = 32$) patients who died and underwent autopsy between a 27-year period were matched to non-RA controls ($n = 32$) of the same age, sex, and level of neurodegenerative proteinopathy. Patients with and without RA had similar prevalence of hypertension, obesity, any cardiovascular disease, and ischemic stroke by medical history. Routine neuropathologic examination was performed at the time of autopsy. Cerebrovascular disease severity was evaluated using modified Kalaria and Strozyk scales. Clinical dementia diagnoses were manually collected from patients' medical records.

Results: Prior to death, 15 (47%) RA patients were diagnosed with dementia; 9 patients in each group (60% and 64%, respectively) had Alzheimer's Disease (AD). The prevalence of cerebral amyloid angiopathy, microinfarcts, infarcts, and strokes was found to be similar between groups. Patients with RA were somewhat more likely to have more severe vascular changes in the basal ganglia by Kalaria scale ($p = 0.04$), but not in other brain areas. There were no significant differences in the presence of large infarcts, lacunar infarcts, and leukoencephalopathy by Strozyk scale.

Conclusions: In this series of autopsies, patients with and without RA had largely similar cerebrovascular pathology when controlling for neurodegenerative proteinopathies, although RA patients had increased cerebrovascular disease in the basal ganglia by Kalaria scale. Additional studies investigating the underlying mechanisms of subclinical worsened small vessel disease within this region in RA patients are needed.