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THE HGF/MET MOTOGENIC SYSTEM IS ACTIVE IN PSEUDOPODIA OF GLIOMA CELLS. Marie E. Beckner*, Naomi R. Agostino and Ian F. Pollack. University of Pittsburgh, Pittsburgh, PA.

Glioma cells and pseudopodia were investigated as a model to study the hepatocyte growth factor (HGF)/scatter factor as a motogen in tumor invasion. HGF and Met are produced by glial cells. Porous filters separated migrated cells and pseudopodia from nonmigrated cells for analysis with densitometry, immunoblotting, and 2-D gels. Multiple cell lines migrated through 8 μm pores of gelatin-coated filters in response to added recombinant human HGF, 2.5 ng/ml. As examples, C6, LN229, and U87 cells demonstrated greater density of Diff Quik stained migrated material in HGF alone, enhanced by adding 0.1% fetal bovine serum (FBS), to 2.15 ± 0.16, 6.30 ± 4.49, and 2.56 ± 0.66 fold greater levels, respectively, than background (no HGF or FBS). U87 pseudopodia, harvested from 3 μm porous filters in the absence of exogenous HGF but with 1% FBS present, demonstrated complete localization of the 69 kDa HGF α-chain (activated) and a 79% increase in the 145 kDa Met compared to whole cells. On 2-D gels, proteins increased in pseudopodia included glycolytic enzymes and annexin 1 which are regulated by HGF (Kaplan O. et al., Neoplasia 2:365-77, 2000; Rothhut B. et al., Cell Mol Life Sci 53;522-6, 1997). Glycogen synthase kinase-3, another target of HGF/Met signaling (Papkoff J, Aikawa M, Biochem Biophys Res Commun 247:851-8, 1998) demonstrated greater phosphorylation in pseudopodia. Nuclear migration of U87 cells, undetected through 3 μm pores at 4-6 h without exogenous HGF, began at 4 h in its presence. HGF's signaling network can be analyzed in glioma pseudopodia.

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EGR-1 REGULATES HYPOXIA-INDUCED TISSUE FACTOR EXPRESSION BY GLIOBLASTOMA. Yuan Rong, Ruo Pan Huang, Donald L. Durden, Erwin G. Van Meir and Daniel J. Brat*. Emory University School of Medicine, Atlanta, GA.

The development of pseudopalisading necrosis in glioblastoma is believed to be critical for rapid growth. We have proposed that intravascular thrombosis and subsequent vaso-occlusion promote pseudopalisade formation and the ensuing hypoxia-induced angiogenic cascade. Tissue factor (TF), the main cellular initiator of blood coagulation, is overexpressed in astrocytomas and may contribute to thrombosis. We previously have shown that tumor hypoxia strongly upregulates TF and promotes plasma clotting, but mechanisms remain undefined. Here, we have investigated the role of early growth response gene product-1 (Egr-1) and hypoxia-inducible factor (HIF) in the hypoxic regulation of TF in GBM cells in vitro. Hypoxia (1% O_2) led to increased Egr-1 mRNA and protein expression within 1h in 23.11 glioma cells, preceding the hypoxic upregulation of TF (6-8 hrs). Overexpression of Egr-1 following cDNA transfection caused upregulation of TF under normoxia (21% O_2), whereas siRNA directed at *Egr-1* strongly down regulated hypoxia-induced TF expression. Hypoxia led to increased nuclear localization of Egr-1 as seen by immunofluorescence and gel shifting experiments demonstrated hypoxia-induced binding of nuclear proteins to the TF promoter in the region of the Egr-1 binding site. To investigate if HIF-1α also regulates TF expression under hypoxia, we used U251 glioma cell lines stably transfected with or without a *HIF-1*α siRNA expression vector. We found that *HIF-1*α mRNA silencing did not affect TF expression under hypoxia while it significantly inhibited both HIF-1α expression and VEGF secretion. We conclude that hypoxic upregulation of TF in GBM cells depends on Egr-1 and is largely independent of HIF-1α.

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IDENTIFICATION OF PHENOTYPICAL CHARACTERISTICS OF INVASIVE ENDOTHELIAL CELLS IN GLIOMA NEOVASCULARIZATION. Ping-Pin Zheng, M. van der Weiden and Johan M. Kros*. Erasmus Medical Centre, Rotterdam, The Netherlands.

Since most gliomas show signs of extensive neovascularization, these tumors are suitable targets for studies in neoplastic neovascularization. Podosomes are highly dynamic, actin-rich adhesional structures, characteristic of motile cells. They facilitate cell migration by modulation of the cell adhesion to the extracellular matrix (ECM), by expressing of modified adhesion structures and degrading of the ECM components by active secretion of proteases. So far, in vivo formation of podosomes in endothelial cells (ECs) has not yet been investigated. In this study, we compared morphological parameters of cell motility, viz., the cell shape, structures of F-actin and focal adhesions (FAs) in ECs of glioma vasculature with those of normal quiescent ECs and motile cells. Targetted were individually deposited ECs or endothelial precursor/-progenitor cells (EPCs), non-canalized endothelial cell chains (NCECCs), either shed from vessel walls or mobilized from bone marrow, and sprouting vessels, present in glioma tissue sections. By double and triple immunofluorescence labeling and laser scanning confocal microscopy (CLSM) the disassembly of FAs and remodeling of F-actin with the formation of podosome-like structures were observed. The ECs often show alterations in cell shape, such as cell enlargement or elongated protrusions, multinucleation and free fragments or cell rounding, witnessing the motility of these cells. Such phenotypical alterations are compatible with the notion that quiescent ECs are activated and move away from their initial location, contributing to the ubiquitous neovascularization in gliomas. Our data are indicative of the activation of EC motility being a crucial event during glioma neovascularization.

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VASCULAR ENDOTHELIAL GROWTH FACTOR UPREGULATES CXCR4 IN GLIOMAS: IMPLICATIONS FOR GLIOMA ASSOCIATED ANGIOGENESIS. Li Lan, Elizabeth Newcomb, M Aktar Ali, Eugene Lukyanov and David Zagzag. New York University School of Medicine, New York, NY.

Stromal cell-derived factor-1 (SDF-1) α, also known as CXCL12, is the only ligand of the chemokine receptor CXCR4. SDF-1α and CXCR4 are required for normal embryonic development of the nervous, hematopoietic, and cardiovascular systems. SDF-1α has angiogenic activities *in vitro* and *in vivo, e.g* SDF-1 is chemotactic for endothelial cells. Previously we analyzed the expression SDF-1α, CXCR4 and HIF-1α in a series of gliomas (JNEN, 2004 63(5):513). Hyperplastic blood vessels, compared with non-hyperplastic ones were strongly positive for SDF-1α. CXCR4 was consistently found in areas colocalizing with HIF-1α expression, suggesting that CXCR4 expression is likely to be determined at least in part by tissue oxygenation. Upregulation of HIF-1α in gliomas is associated with concomitant upregulation with one of his target genes vascular endothelial growth factor (VEGF). To further investigate the role of SDF-1α and CXCR4 in human glioma associated angiogenesis, we tested the effect of hypoxia and VEGF on the expression of SDF-1α and CXCR4 in human brain microvascular endothelial cells (HBMECs) *in vitro*. We show that the level of CXCR4 mRNA expression increased in HBMECs after 6 hours exposure to VEGF and was maintained up to 48 hours. In contrast, the level of SDF-1α mRNA expression was not changed over the same time interval. Furthermore, we show that both SDF-1α and CXCR4 mRNA expression were significantly induced after 12 hours exposure to hypoxia. These data suggest that CXCR4/SDF-1α axis play an important role in glioma-associated angiogenesis, which could provide potential targets for chemotherapy in glioma.

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NESTIN EXPRESSION IN GLIOMAS AND THE CONCEPT OF TUMOR STEM CELLS. Davide Schiffer*, Valentina Fiano and Chiara Ghimenti. University of Turin, Foundation Policlinico di Monza.

In malignant gliomas, clonogenic, multipotent, neurosphere forming cells are supposed to derive from primary or transformed neural stem cells. In tumors their identification has been made by the surface marker CD133, whereas nestin, vimentin and GFAP expression are discussed in relation with the progenitor status of the supposed cells of origin. Whether tumor stem cells represent highly undifferentiated neural cells or extremely de-differentiated clones with accumulated mutations must be discussed considering the involvement in malignant gliomas of the same molecular pathways regulating differentiation/de-differentiation during cytogenesis. We wanted to verify how nestin expression, typical of stem/progenitor cells, can contribute to this problem. In 50 brain tumors, including astrocytomas grade II-IV, pilocytic astrocytomas, oligodendrogliomas and ependymomas, nestin expression was found to roughly overlap with that of vimentin and GFAP by immunohistochemistry and Western blot. Highly expressed in glioblastomas, pilocytic astrocytomas and ependymomas, mainly in relation with the extension of cytoplasms, poorly expressed in astrocytomas, it was negative in oligodendrogliomas with the exception of some round cytoplasms. In glioblastomas, areas of densely packed small cells with hyperchromatic nuclei and a high number of mitoses, once considered as primitive neuroepithelial or de-differentiated or stem cells, responsible for invasion and recurrence, were negative for the three intermediate filaments. Therefore, they more likely represent a transformed phenotype.

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NOTCH3 SIGNALING INITIATES CHOROID PLEXUS TUMOR FORMATION. Charles G. Eberhart, Louis Dang, Xing Fan, Aneeka Chaudhry and Nicholas Gaiano. [1]Johns Hopkins University School of Medicine, Baltimore, MD.

Objective: To determine the effects of Notch3 activation on murine brain development. **Results:** We demonstrate that constitutively active Notch3 signaling causes the formation of choroid plexus tumors in mice. The mammalian Notch1 and Notch2 receptors regulate specification and proliferation of neural progenitor cells. Notch2 signaling also promotes the growth of progenitor cell like embryonal brain tumors. Notch3 is expressed in fetal nervous system progenitor cells, but little is known about its role in brain development or the neoplastic transformation of neural cells. We found that introduction of activated Notch3 into the periventricular cells of embryonic day 9.5 mice was sufficient to initiate formation of choroid plexus tumors. Tumors arose in the 4th ventricles in 83% of animals and were associated with hydrocephalus. They were microscopically highly similar to choroid plexus papillomas in humans, with an ongoing proliferation rate of 4-6%. Signs of Notch pathway activity were also present in human choroid plexus lesions. Notch receptor mRNA levels in human choroid plexus papillomas were elevated over those in non-neoplastic choroid plexus, with Notch2 overexpressed approximately 500-fold in one tumor. Nuclear Notch protein was also identified immunohistochemically in human lesions, suggesting the pathway had been activated. **Conclusions:** Our findings indicate that activated Notch3 can act as an oncogene in the developing brain, and link the Notch pathway to human choroid plexus tumor pathogenesis.

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CONSTITUTIVE SIGNALING BY NEUREGULIN-1 AND ERBB KINASES PROMOTES MITOGENESIS IN A TRANSGENIC MOUSE MODEL OF MALIGNANT PERIPHERAL NERVE SHEATH TUMORIGENESIS. Steven L. Carroll*, Mark S. Stonecypher and Stephanie J. Byer. University of Alabama School of Medicine, Birmingham, AL.

We have previously shown that human malignant peripheral nerve sheath tumors (MPNSTs) express neuregulin-1 (NRG-1) growth factors and their erbB receptors and that the proliferation of cultured human MPNST cell lines is dependent on NRG-1/erbB signaling. To further test the hypothesis that NRG-1/erbB signaling promotes MPNST tumorigenesis, we produced transgenic mice expressing the NRG-1 isoform GGFβ3 in Schwann cells (P_0-GGFβ3 mice). 70% of P_0-GGFβ3 mice develop MPNST-like neoplasms, both on outbred (C57BL/6 x SJL) and inbred (C57BL/6) genetic backgrounds. Analyzing 18 Schwann cell, neural crest, muscle and neural markers in 19 permanent cell lines derived from tumors arising in P_0-GGFβ3 mice, we found that these lines uniformly express Schwann cell markers and variably express markers of other lineages. The growth of murine MPNST cell lines is anchorage-independent in soft agar and these lines form subcutaneous masses when allografted into SCID or NIH III mice. Murine MPNST cell lines all coexpress multiple NRG-1 isoforms and varying combinations of erbB kinases. ErbB kinases expressed by these lines are constitutively tyrosine phosphorylated. Treatment with the erbB inhibitors PD168393 and PD158780 markedly reduces DNA synthesis in murine MPNST cells, demonstrating their dependence on erbB signaling. Treatment with erbB4 soluble receptor (erbB4-F_c), but not with erbB2-F_c or human F_c, likewise inhibits human and murine MPNST cell mitogenesis. Our findings argue that NRG-1/erbB signaling promotes neoplastic Schwann cell mitogenesis in MPNSTs and suggests that P_0-GGFβ3 mice will be useful models for evaluating therapeutic regimens targeting NRG-1/erbB signaling in human MPNSTs. Supported by NINDS grant R01 NS048353.

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INTERACTION BETWEEN ALPHA9BETA1 INTEGRIN AND TENASCIN-C CONTRIBUTES TO LEPTOMENINGEAL ADHERENCE OF MEDULLOBLASTOMA CELLS. Sidney E. Croul[1]*, Izabela Staniszewska[2], Krzysztof Reiss[2], Kamel Khalili[2] and Cezary Marcinkiewicz[2]. [1]Drexel University College of Medicine and [2]Temple University, Philadelphia, PA.

The cellular mechanisms which govern subarachnoid growth of medulloblastomas are not well understood. To characterize the integrins and matrix molecules which are involved in this process we have used assays which quantitate the adhesion of cells to immobilized matrix components, immobilized antibodies to specific integrin subunits, or snake venom disintegrins. When defined matrix proteins were tested in quantitative adherence assays, 50% of cells adhered to the matrix from a glial feeder layer, while 15% adhered to fibronectin, 15% to vitronectin and 5% to collagen type IV. By ELISA, the glial matrix is predominately composed of tenascin –C and to lesser extent collagen IV, vitronectin and laminin. An in vitro adhesion assay with immobilized monoclonal anti-integrin antibodies demonstrates greatest adherence of D283 cells to antibodies to the beta 1 subunit and to the alpha9beta1 integrin with lesser contributions by the alpha1 and alpha6 subunits. Immobilized snake venom disintegrins show greatest adherence to MLD containing disintegrins that have previously been characterized as potent inhibitors of alpha9beta1 integrin (BG9 and VLO5). Monoclonal antibodies to the alpha9beta1 integrin and to the beta1 integrin subunit potently inhibit adhesion of the D283 cells to the glial matrix as do the MLD containing disintegrins BG9 and VLO5. These results suggest that D283 cells utilize the alpha 9beta1 integrin to interact with tenascin –C. This may contribute to the mechanism by which medulloblastomas adhere to the leptomeninges during subarachnoid dissemination.

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GERSTMANN-STRÄUSSLER-SCHEINKER DISEASE ASSOCIATED WITH THE PRNP A117V-129V MUTATION: NEUROPATHOLOGY OF AN ASYMPTOMATIC GENE CARRIER AND FIVE CLINICALLY AFFECTED INDIVIDUALS FROM A FAMILY. Masaki Takao[1,2]*, Piccardo Pedro[1,3], Martin R. Farlow[1], Jill R. Murrell[1], Frederick W. Unverzagt[1], Keiji Yamaguchi[1], Bradley S. Glazier[1], Francine Epperson[1], Eileen Bigio[4], Charles DeCarli[5] and Bernardino Ghetti[1]. [1]Indiana Alzheimer Disease Center, Department of Pathology & Laboratory Medicine, Department of Neurology, Department of Psychiatry, Indiana University School of Medicine, Indianapolis, IN. [2]Mihara Memorial Hospital, Isesaki, Gunma, Japan. [3]Center for Biologics Evaluation and Research, Food and Drug Administration, Rockville, MD. [4]Cognitive Neurology & Alzheimer's Disease Center, Department of Pathology, Northwestern University Feinberg School of Medicine, Chicago, IL. [5]Alzheimer's Disease Center, Department of Neurology, University of California, Davis, Sacramento, CA.

Gerstmann-Sträussler-Scheinker disease (GSS) associated with the A117V *PRNP* gene mutation has been reported in at least eight families. The aim of this study is to extend the characterization of the neuropathologic phenotype associated with this disorder by studying six individuals, one asymptomatic and five symptomatic, from the same American family. The affected individuals' mean age at onset of symptoms is 41±6 y (33-49 y) and mean age at death is 45±7 y (38-55 y). The asymptomatic gene carrier died of heart failure at age 50. The five affected individuals developed dysarthria as an initial symptom followed by mild cognitive dysfunction, ataxia and extrapyramidal symptoms such as rigidity, masked face and dystonia as well as dementia. Neuropathologically, PrP-immunopositive plaques were seen in the cerebral and cerebellar cortices, hippocampus, amygdala as well as basal ganglia. Most of the plaques had multi-centric amyloid cores. In the cerebral cortex and hippocampus, several plaques did not have any amyloid core, but appeared as round, eosinophilic structures similar to cotton wool plaques (CWPs); however, they were immunonegative for Aβ protein. Diffuse PrP-immunopositive deposits and vacuolar changes were present in the upper cortical layers of the cerebrum. Although neuronal loss occurred in the substantia nigra, no PrP-immunopositive deposits were observed. In the asymptomatic carrier, PrP-immunopositive deposits were seen only in the cerebellar cortex. The present study shows that in GSS associated with the A117V *PRNP* mutation, PrP deposits are either diffuse, multicentric or similar to CWPs. P30 AG10133, JSPS 15600226.

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COULD AXONAL DEGENERATION ASSOCIATED WITH HIV INFECTION LEAD TO AD-LIKE PATHOLOGY? Cristian L. Achim. Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA.

We have previously showed that brain b-amyloid deposition is a common finding in HIV patients and it directly correlates with age. We hypothesize that in long-term survivors on HAART, beta-amyloid deposition may become a prevalent pathologic event that could contribute to the development of neurocognitive impairment at younger ages. Furthermore, our most recent preliminary data suggest that Tau aggregation in the HIV brain may also be a more frequent event than previously thought. We have now strong evidence that axonal pathology in chronic HIV infection may be associated with changes in the chaperone function of brain immunophilins. To test in vivo our hypotheses we have initiated a clinical brain imaging study for beta-amyloid in HIV patients on HAART, with or without cognitive impairment, using a PET ligand developed at the University of Pittsburgh. We are also currently expanding our autopsy studies of HIV brains to determine the extent, regional and cellular distribution and co-localization of b-amyloid deposition, Tau aggregation and the immunophilin response. Based on the preliminary results we propose that beta-amyloid deposition and axonal pathology may increase in long-term surviving HIV patients and could represent the pathologic substrate for minor cognitive impairment. Early diagnosis and better understanding of the mechanisms of disease may lead to the development of potentially neuro-protective therapies both in the HIV and pre-AD patient populations. **Acknowledgements:** Drs. Harry V. Vinters (UCLA), Eliezer Masliah (UCSD), Chester Mathis (UPMC PET Center), Douglas A. Green (UPSOM, MSTP), Mihaela Avramut (UCSF). MH072529 to CLA, HNRC Developmental Grant to DAG.

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CONCOMITANT ALZHEIMER'S DISEASE AND FRONTOTEMPORAL DEMENTIA-MOTOR NEURON DISEASE SUBTYPE: A COMMON NEUROPATHOLOGIC PHENOMENON. Marla Gearing, James J. Lah and Allan I. Levey. Emory University, Atlanta, GA.

Numerous reports indicate that pathologic changes of Alzheimer's disease (AD) and other neurodegenerative diseases often occur concomitantly. In the present study, we examined all of the AD cases accessioned over the previous three years to assess the degree of neuropathologic overlap between AD and frontotemporal dementia-motor neuron disease subtype (FTD-MND). Adjacent sections of hippocampus and entorhinal, frontal and temporal cortices were immunohistochemically labeled with antibodies to ubiquitin and tau, and the frequency of ubiquitin-positive, tau-negative inclusions in neurons and neurites in superficial layers of cortex and hippocampal dentate gyrus was scored semiquantitatively. Given the challenge inherent in distinguishing the inclusions of FTD-MND from those in AD, we opted to use conservative criteria for the neuropathologic diagnosis of FTD-MND, and required the presence of inclusions in both hippocampal dentate neurons and frontal or temporal cortex. We found concomitant FTD-MND pathology in 15 (33%) of 46 AD cases examined. Interestingly, the AD+FTD-MND cases were significantly older than cases with either FTD-MND or AD alone (AD+FTD-MND: 85.5 years, AD: 77.8 years, FTD-MND: 65.3 years; $p < .001$). The degree of overlap between AD and FTD-MND was surprising. Clearly, identification of the specific protein aggregating in FTD-MND is critical and will permit more reliable diagnosis of FTD-MND both in isolation and in conjunction with other neurodegenerative diseases such as AD. In addition to a potential contribution to the variability of clinical symptoms observed in AD, the overlap between AD and FTD-MND could also have pharmaceutical implications for the treatment of these disorders. Supported by AG10130, ES12068.

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NOVEL UBIQUITIN BRAIN PATHOLOGY IN FRONTOTEMPORAL DEMENTIA WITH INCLUSION BODY MYOPATHY AND PAGET'S DISEASE. Mark S. Forman[1]*, Ian R. Mackenzie[2], William R. Markesbery[3], Eric Swanson[1], Nigel J. Cairns[4], Philip J Boyer[5], Bharvati S. Jhaveri[6], Jason H. Karlawish[1], Daniel W. McKeel[4], Alan Pestronk[4], Giles DJ. Watts[7], Charles D. Smith[3] and Virginia E. Kimonis[7]. [1]University of Pennsylvania, Philadelphia, PA. [2]University of British Columbia, Vancouver, BC. [3]University of Kentucky, Lexington, KY. [4]Washington University, St. Louis, MO. [5]University of Texas Southwestern Medical School, Dallas, TX. [6]St. John's Hospital, Springfield, IL. [7]Harvard Medical School, Boston, MA.

Inclusion body myopathy with Paget's disease of bone and frontotemporal dementia (IBMPDFD) is a rare progressive autosomal dominant disorder. Recently, causative mutations were identified in affected individuals within *valosin-containing protein* (*VCP*), a member of the AAA-ATPase gene superfamily. Neuropathology associated with the clinical syndromes of frontotemporal dementia (FTD) is heterogeneous including tauopathies and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U). However, there is limited information on the CNS pathology associated with IBMPDFD. To characterize the neuropathology of IBMPDFD, we analyzed the brains of 7 patients from 4 families with VCP mutations. Five patients were diagnosed clinically with FTD, whereas 2 manifested only IBM or IBM plus PD. FTLD-U pathology was identified in all 5 patients with FTD and in one non-demented patient. One FTD patient also had intermediate probability AD. The FTLD-U pathology was distinctive with a relative abundance of neuronal intranuclear inclusions and dystrophic neurites and few neuronal cytoplasmic inclusions, compared to a large series of sporadic and familial FTLD-U. The inclusions did not stain for tau, -synuclein, and neurofilament or with multiple antibodies to VCP. While the ubiquitin inclusions were abundant throughout the neocortex, pathology was less robust in limbic and subcortical nuclei and the dentate gyrus was spared. VCP is associated with a variety of cellular activities including regulation of the ubiquitin-proteasome system. Thus, the identification of FTLD-U pathology in IBMPDFD suggests that VCP mutations may directly impair ubiquitin-based degradation pathways that are implicated in a variety of neurodegenerative disorders including amyotrophic lateral sclerosis and Parkinson's disease.

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SEVERE FRONTOTEMPORAL GLIAL PATHOLOGY AND MILD HIPPOCAMPAL DEGENERATION ASSOCIATED WITH THE P301L MAPT MUTATION: STUDY OF A PREVIOUSLY UNREPORTED FAMILY. Salvatore Spina[1], Jill R. Murrell[1], Marla Gearing[2], Allan Levey[2], Suzanne Mirra[3], Keiji Yamaguchi[1], Bernardino Ghetti[1]* and James Lah[2]. [1]Indiana Alzheimer Disease Center, Indiana University, Indianapolis, IN. [2]Emory University, Atlanta, GA. [3]The State University of New York, New York, NY.

P301L is the commonest *MAPT* mutation associated with frontotemporal dementia. Perinuclear deposits of tau in the form of rings or round bodies, in CA1 neurons, dentate gyrus, temporal and entorhinal cortices have been reported as a consistent neuropathologic finding associated with the P301L *MAPT* mutation. The proband, a 48-year-old woman, presented with progressive apraxia of speech. Four years later she developed memory and attention impairment. At age 53, her speech was unintelligible. She became emotional labile, fell repeatedly, and her self-care was reduced. Subsequently, she developed frontal release signs, rigidity, dystonia and dysphagia. She died at age 55. A neuropathologic examination revealed severe glial pathology consisting of numerous tau-immunoreactive tufted astrocytes and astrocytic plaques in the neocortex. Perinuclear deposits in the dentate gyrus were rarely observed. The CA1 contained only a few pre-tangles as well as mild neuronal loss. Genetic analysis revealed the presence of a P301L mutation in *MAPT*. A brother of the proband started presenting a progressive irritability at age 57. He became obsessive, inappropriate and distractible. Abstract reasoning, verbal fluency and naming declined. At age 59, he became dependent for activities of daily living and presented dysphagia, hyperphagia, sleep abnormalities, frontal signs, incoordination, altered pursuit and shuffling gait. His memory was relatively preserved and his MMSE score was 24/30. A sister of the proband was recently diagnosed with frontotemporal dementia. Their mother and other maternal relatives were diagnosed with dementia. Our findings emphasize the clinical and neuropathologic phenotypic heterogeneity associated with the P301L *MAPT* mutation. P30 AG10133.

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MESIAL TEMPORAL SCLEROSIS IS ASSOCIATED WITH LEWY BODIES IN ALZHEIMER'S DISEASE. Amy Y. Yen, Oscar L. Lopez, James T. Becker and Ronald L. Hamilton*. University of Pittsburgh, Pittsburgh, PA.

Cerebral Lewy bodies (LB) are common in Alzheimer's Disease (AD), and can be detected in 40-60% of cases using alpha-synuclein immunohistochemistry. Mesial Temporal Sclerosis (MTS) (which includes pure hippocampal sclerosis) is often associated with epilepsy or global ischemia, but can be a primary dementing disorder (that is, without significant AD pathology). However, some AD cases (without a history of cardiac arrest or seizures) have MTS. We examined 278 cases of AD that had been part of the Pittsburgh ADRC cohort from 1986-2004 to determine if there was an association between MTS and LB in AD. All AD cases were clinically diagnosed as "Probable AD" using NINCDS-ADRDA criteria and met CERAD criteria for the neuropathological diagnosis of "definite AD." Braak tangle stage was done using Bielschowsky stain and LB were detected using monoclonal antibody to alpha-synuclein (Zymed) with protease pre-treatment. 149/278 had LB (53.6%) and 33/278 had MTS (11.9%). Using Student's t-test, AD+MTS showed lower brain weight ($p = 0.00046$) and greater age ($p = 0.002$), than AD. There was no difference in Braak stage between AD and AD+MTS (χ^2 test, $p = 0.74$). There was also no association of MTS with infarcts (χ^2 test, $p = 0.47$). Of the 33 AD cases with MTS, 26/33 (78.8%) had LB (χ^2 test, $p = 0.002$). There was no significant difference in the LB scores between AD+LB and AD+LB+MTS cases (student t-test, $p = 0.58$). This study indicates that MTS in AD is found in older patients and is usually associated with LB.

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OCCURRENCE OF A-SYNUCLEIN DEPOSITION IN FOUR-REPEAT TAUOPATHIES VERSUS ALZHEIMER DISEASE. Salvatore Spina, Pedro Piccardo, Masaki Takao, Keiji Yamaguchi, Tatiana Foroud and Bernardino Ghetti*. Indiana University, Indianapolis, IN.

The accumulation of misfolded proteins occurs in numerous neurodegenerative disorders. It has been postulated that α-synuclein and tau act synergistically in the formation of intracellular protein aggregates. The aim of our study was to determine how frequently α-synuclein-immunopositive deposits are seen in pathologically-confirmed cases of progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), argyrophilic grain disease (AGD) and compare it to that of Alzheimer disease (AD). Tau deposits in PSP, CBD and AGD contain predominantly four-repeat tau isoforms, whereas in AD they contain both three- and four-repeat tau isoforms. We analyzed 27 cases of PSP, 4 of CBD, 3 of AGD and 277 of AD. A-synuclein and tau deposition was determined by immunohistochemistry. A-synuclein immunoreactive deposits were observed in two cases of PSP, none of the CBD and AGD cases and 66 cases of AD. Overall α-synuclein deposits were noted in 5.9% of the four-repeat tauopathies and 23.7% of the three- and four-repeat tauopathies. As our results confirm data previously reported in the literature, it remains to be determined whether the greater frequency of α-synuclein deposits in AD compared to that in four-repeat tauopathies may be influenced by the nature of tau deposits or by the presence of Aβ pathology in AD. Supported by P30 AG10133, R01 NS37167, U24 AG21886.

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NEUROPATHOLOGY OF FAMILIAL PARKINSON'S DISEASE DUE TO *LRRK2* MUTATIONS. Dennis W. Dickson, Owen A. Ross and Matthew J. Farrer. Mayo Clinic, Jacksonville, FL. (Sponsored by Steven G. Younkin*.)

Background: Mutations in a gene on chromosome 12, *LRRK2*, may be the most common cause of familial Parkinson's disease (PD). The neuropathology of reported cases is heterogenous, including Lewy body (LBs), neurofibrillary tangles (NFTs) or nonspecific neuronal loss and gliosis, depending upon the individual and family. One of the most common pathogenic mutations in *LRRK2* is a G2019S substitution. It has been reported in up to 5% of familial and 1% of sporadic PD. **Objectives:** To determine the frequency of *LRRK2* G2019S in a collection pathologically confirmed degenerative disorders and control brains and to assess the influence of the mutation on neuropathology. **Methods:** DNA was extracted from 1400 brains and screened for the 6055G>A (G2019S) mutation with Taqman Probe Chemistry on an ABI7900 sequenceer. Positive and negative controls were used. **Results:** We identified twelve G2019S-positive cases, with material available for neuropathologic review on eight of the cases. All but one of the eight cases had LBs, ranging from brainstem-predominant to diffuse cortical type. One case of frontotemporal degeneration with ubiquitin inclusions was also postive for the mutation. **Conclusion:** This is the first comprehensive screening for G2019S in a brain bank of diverse neurodegenerative disorders. The presence of LBs in 90% of the mutation carriers indicates that mutations within the kinase domain of the *LRRK2* protein contribute to alpha-synuclein pathology. The absence of LBs in some cases with *LRRK2* mutations may reflect reduced penetrance of the mutation or pathological pleomorphism.

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UPREGULATION OF THE SIGNALING ADAPTOR PROTEIN SHCA IN ACTIVATED MICROGLIA OF MULTIPLE SCLEROSIS PLAQUES. Brett S. Stetka and James W. Mandell*. University of Virginia, Charlottesville, VA.

Growing evidence implicates MAP kinase pathways in initiating and maintaining reactions of macrophages, microglia and astroglia, prominent players in multiple sclerosis (MS) plaques. ShcA is an adaptor protein linking surface receptors to MAPK signaling pathways, whose expression is normally restricted to neural development. Recent gene array studies identified ShcA as an upregulated gene in MS plaques. Objective: to confirm ShcA upregulation at the protein level and to determine the cellular identity of ShcA-expressing cells in MS plaques. Immunohistochemistry for ShcA, GFAP, CD68, and HAM68 was performed in on sections from normal (n = 5) and MS plaque (n = 10) white matter tissues. ShcA immunoreactivity was low normal adult white matter but greatly increased at the periphery of chronic-active MS lesions. The astrogliotic centers of plaques were immunonegative. The majority of the ShcA-positive cells were CD68-positive and HAM68-negative, suggesting their identity as activated microglia. To test whether ShcA expression was regulated in a cell culture model of microglial activation, primary mouse microglial cultures were treated with lipopolysaccharide (4 μg/ml) or vehicle for 24 hr. Double-label immunofluorescence confirmed LPS-induced upregulation of ShcA immunoreactivity in IB4 lectin-positive microglia after LPS treatment. We conclude that ShcA expression is increased in chronic-active MS plaques, largely associated with activated microglia. Testable functions of ShcA in activated microglia include potentiation of migratory and/or phagocytic activity in the setting of myelin damage. Supported by NS047378-01(JWM). MS brain tissue was obtained from Walter W. Tourtellotte, MD, PhD, UCLA Human Brain and Spinal Fluid Resource Center.

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MONOCLONAL ANTIBODIES (mAbs) TO DISTINCT REGIONS OF MYELIN PROTEOLIPID PROTEIN (PLP) REACT WITH NEURONS OF MANY VERTEBRATE SPECIES: POTENTIAL IMPLICATIONS FOR MULTIPLE SCLEROSIS (MS). Raymond A. Sobel[1]*, Jayagopala Reddy[2,3], Vijay K. Kuchroo[2,3], Marjorie B. Lees[3,4]* and Edward Greenfield[2,3,5]. [1]VA Health Care System Palo Alto and Stanford University School of Medicine, Stanford CA. [2]Brigham & Women's Hospital and [3]Harvard Medical School, Boston, MA. [4]Shriver Center, Waltham and University of Massachusetts Medical School, Worcester, MA. [5]Millennium Pharmaceuticals, Cambridge, MA.

The proteolipid gene family (pgf) includes the PLP/DM20 and M6 genes. PLP, the major protein of mammalian CNS myelin, is a polytopic membrane protein and a potential MS autoantigen. M6a/b are neuronal membrane glycoproteins involved in Ca^{++} channel function; an anti-M6 mAb impairs neuronal growth and development in vitro. Human M6a/b and PLP have relatively high degrees of amino acid identity in PLP regions 178-191, 200-219 and 262-276. To determine whether anti-PLP antibodies (Abs) recognize evolutionarily conserved pgf epitopes, CNS sections from representative vertebrates were immunostained with mAbs specific for PLP residues 50-69, 120-139, 178-191, 200-219 and 262-276. All species tested had Luxol fast blue+ CNS myelin. All mAbs immunostained mammalian and avian CNS myelin. The mAbs to highly conserved PLP regions stained amphibian, teleost and elasmobranch CNS myelin; there was less consistent myelin staining with mAbs to less conserved regions. In these vertebrates, DM-20, the PLP isoform which lacks residues 116-150, is the major CNS myelin protein; the anti-120-139 mAb did not stain their myelin. Unexpectedly, the anti-178-191 mAb additionally stained some mammalian and avian neurons; the anti-178-191 and -262-276 mAbs stained most amphibian, teleost and elasmobranch CNS neurons and neuropil. These results indicate that anti-PLP mAbs recognize conserved pgf epitopes in both CNS myelin and neurons. In MS patients with anti-PLP Abs, cross-recognition of neurons might have inhibitory physiologic effects similar to those of the anti-M6 mAb. Thus, anti-PLP Abs associated with demyelination in MS may impair neuronal function and differentiation through pgf epitope recognition.

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MYOINOSITOL ADMINISTRATION REDUCES DEMYELINATION AFTER RAPID CORRECTION OF HYPONATREMIA. S. M. Silver[1], B. Schroeder[1], R. H. Sterns[1] and A. M. Rojiani[2]. [1]Rochester General Hospital, Rochester, NY. [2]University of South Florida, Tampa, FL.

When chronic hyponatremia is rapidly corrected, re-accumulation of brain organic osmolytes is delayed and brain cell shrinkage and demyelination occurs, leading to the osmotic demyelination syndrome. We hypothesized that treatment with myoinositol, a major organic osmolyte, could decrease demyelination in this setting. Severe hyponatremia was induced in 24 adult male rats by administration of vasopressin and intravenous infusion of dextrose and water. Sixty-four hours after induction of hyponatremia, all animals underwent rapid correction of hyponatremia with infusion of hypertonic saline over 4 hours, increasing the serum sodium from 106 to 124 mM. The animals were also given in a blinded fashion either myoinositol or mannitol (control) intravenously beginning 20 minutes prior to correction and continuing for 24 hours. Serum sodium concentrations were equivalent in both groups at all time points. Animals were sacrificed 96 hours after correction of hyponatremia was begun, or earlier if they appeared moribund. Myoinositol treated animals had significantly fewer demyelinating brain lesions than mannitol treated (2.3 ± 1.1 vs. 6.4 ± 1.4 lesions/brain, $p < 0.03$), and had a significantly lower pathologic score based on number and severity of demyelinating lesions (3.3 ± 1.8 vs. 10.1 ± 2.7 score/brain, $p < 0.03$). We conclude that myoinositol administration reduces myelinolysis after rapid correction of chronic hyponatremia in rats.

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THE CONCURRENCE OF INFLAMMATORY DEMYELINATION AND ANAPLASTIC ASTROCYTOMA IN A SINGLE BRAIN BIOPSY. Shanu F. Roemer, Bernd W. Scheithauer*, Bradley Erickson and Claudia F. Lucchinetti. Mayo Clinic College of Medicine, Rochester, MN.

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system characterized by lymphohistocytic inflammation, demyelination and variable remyelination, relative axonal preservation and astrogliosis. The latter is subacute and often accompanied by Creutzfeld cells. Anaplastic astrocytoma (AA) features cytologically malignant cells undergoing mitotic activity. Since MS occasionally presents with clinical and radiographic features indistinguishable from those of a brain tumor ("tumefactive MS"), a biopsy is needed to secure a diagnosis. The concurrence of MS and astrocytoma is a rare phenomenon. We report a case of a 49 year old woman who underwent biopsy of what clinically and radiologically appeared to be a brain tumor. The MRI showed a complex enhancing mass with a ring component associated with central T1 hypointensity, a rim of T2 hypointensity, and a contiguous more solid enhancing component. On histologic examination, a small portion of the specimen exhibited all the characteristics of AA. In addition, the sample contained separate fragments of typical demyelinating disease. Routine histology (H&E), myelin stains (LFB-PAS) and immunostains for macrophages (KP-1) and axons (neurofilament protein) clearly distinguished the two processes. The architecturally solid tumor showed near total loss of axons whereas the demyelinating zone showed their relative preservation. This observed concurrence of AA and MS raises questions regarding the genesis of the two processes, i.e whether they present a coincidental finding or, alternatively, share a pathogenic mechanism.

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PRESENCE AND DISTRIBUTION OF DENDRITIC CELLS IN INFLAMMATORY DISEASES OF THE CENTRAL NERVOUS SYSTEM. Billy B. Teng and Katerina Dorovini-Zis*. Vancouver General Hospital and University of British Columbia, Vancouver, BC, Canada.

Dendritic cells (DCs) are potent antigen-presenting cells responsible for the initiation of adaptive immunity through their ability to prime naïve T-cells and to stimulate antigen-primed T-cells. Their contribution to central nervous system (CNS) inflammation, however, has not yet been characterized. Recent studies have observed DCs in the meninges and choroid plexus in the normal human CNS. In this study, we used an immunohistochemical approach to investigate the presence and distribution of DCs in inflammatory CNS lesions of various etiologies. Formalin-fixed, paraffin-embedded sections of brain and spinal cord were incubated with monoclonal antibodies against the DC-specific markers DC-SIGN and fascin, as well as against MHC class II and CD40. In the normal brain, a small number of DCs were present in the meninges but absent in the parenchyma. Prominent perivascular cuffs of DCs were observed in chronic granulomatous inflammation; vasculitides; viral infections; toxoplasmosis; fungal meningoencephalitis; acute infarcts; primary and secondary tumors; multiple sclerosis; and brain abscess. DCs were also detected in the CNS parenchyma in the vicinity of the perivascular cuffs; this was especially apparent in chronic granulomatous inflammation and tumors. DCs were absent in acute bacterial meningitis and ALS. The presence of DCs in CNS inflammation suggests a potential role of these cells in antigen presentation and initiation of immune responses in the CNS.

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MONOCYTE ADHESION TO BRAIN MICROVESSEL ENDOTHELIAL CELLS: REGULATION BY ADHESION MOLECULES AND BETA-CHEMOKINES. K. Liu, R. Prameya, R. Shahidi, V. Wu and K. Dorovini-Zis*. Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, Canada.

Influx of monocytes into the brain is a feature of many CNS disorders including trauma, ischemia, infections and autoimmune diseases. The mechanisms that regulate monocyte trafficking across the blood-brain barrier (BBB) remain poorly understood. In this study we investigated the factors that mediate the adhesion of human peripheral blood monocytes to human brain microvessel endothelial cells (HBMEC) in an in vitro BBB model. Primary cultures were grown to confluence in a double chamber chemotaxis system. HBMEC monolayers were used untreated or treated with TNF-α and IFN-γ for 24 hours. Monocytes were placed over HBMEC and incubated for 15 to 60 minutes at 37°C. Cytokine treatment significantly augmented adhesion to HBMEC. Blocking antibodies to CD49d, ICAM-1, VCAM-1 or PECAM-1 decreased the adhesion of monocytes to HBMEC. SEM and confocal microscopy studies suggest that monocyte adhesion occurs mostly at or near interendothelial tight junctions. In order to study the effects of beta-chemokines on monocyte adhesion to cerebral endothelium, CCL2 and CCL3 were placed in the lower chamber to establish concentration gradients. Radiolabeled chemokines diffused across cytokine-activated and to a much lesser extent resting HBMEC monolayers in a time dependent manner and bound to the subendothelial matrix and endothelial cell surface. Concentration gradients of CCL2 and to a lesser extent CCL3 increased the adhesion of monocytes to untreated and cytokine-treated HBMEC. These findings suggest that adhesion molecules/integrin interactions and the beta-chemokines CCL2 and CCL3 play an important role in the recruitment of monocytes across the BBB to sites of CNS inflammation.

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MRI–PATHOLOGY CORRELATIONS IN SPINAL DISC DISEASE. Douglas C. Miller*, Paul Pevsner and Codrin Iacob. New York University School of Medicine, New York, NY.

Back pain from intervertebral disc disease is a common and expensive health care problem. Multiple reports of MRI changes in the spine and paraspinous soft tissues have had limited pathologic correlation. The pathogenesis of pain is poorly understood and the rationale for surgical intervention is often weak. We obtained 129 vertebral columns from adult autopsies, which are being imaged in sagittal orientation; each intervertebral joint is then sectioned sagittally such that each section includes a portion of vertebral body above and below the disc, plus the entire disc and the anterior and posterior paraspinous soft tissues. This report, from the initial 10 specimens so examined, used H&E stained sections. MRI showed anterior and posterior disc protrusions at almost every level examined. Histologically, there was exact correlation with the MRI in virtually all levels examined of each spine. Disc tissue of the annulus fibrosus bulged beyond the anterior and posterior limits of the vertebrae, with upward and downward extensions of fibrocartilaginous tissue over the bone. Posteriorly the annulus fused with the posterior longitudinal ligament. Osteophytes of metaplastic bone arising in the bulging cartilage were common. The PLL contains substantial nerve bundles, which might account for pain with disc bulges without frank herniation of disc fragments into neural foramina. The conventional diagnostic criteria for disc bulges, herniations, and protrusions likely need to be redefined in light of these findings.

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HISTOLOGICALLY UNREMARKABLE DORSAL PREFRONTAL WITE MATTER IN SCHIZOPHRENIA. Andrew J. Dwork[2,3,5], Boro Ilievski[1], Branislav Mancevski[2,3], Matthew Kurzon[2], Tereza Serafimova[4], Iskra Trencevska[2], Gorazd Rosoklija[2,3,4] and John Keilp[2,3]. [1]Institute for Pathology, School of Medicine; and [4]Department of Psychiatry, School of Medicine, "Ss. Cyril & Methodius," Skopje, Macedonia. [2]Department of Neuroscience, New York State Psychiatric Institute; [3]Department of Psychiatry, College of Physicians and Surgeons of Columbia University; and [5]Department of Pathology, College of Physicians and Surgeons of Columbia University, New York, NY.

In vivo imaging studies, particularly diffusion tensor imaging, have detected abnormalities of cerebral white matter in schizophrenia. The current study was undertaken to determine whether there are corresponding histological abnormalities. A secondary goal was to determine whether white matter abnormalities, possibly secondary to vascular disease, are associated with the pronounced cognitive impairment that is common in elderly individuals with schizophrenia. Detailed medical record reviews and neuropathological examinations were performed on 95 subjects with schizophrenia or schizoaffective disorder and 28 subjects without psychiatric disease. A set of histological sections, comprising the dorsal half of the frontal lobe at the level of the rostral tip of the lateral ventricle, was stained for myelin with Verhoeff's stain. Using systematic random sampling of the white matter, over 200 microscopic fields per case were examined at high magnification by a single observer, masked to clinical and demographic information. As expected, lower myelin ratings were associated with cerebrovascular disease and, independently, with increasing age, but not with schizophrenia. Among the schizophrenia cases, there were no significant differences in myelin ratings between those with (N = 65) and without (N = 27) definite cognitive impairment. We conclude that white matter abnormalities in schihzophrenia either spare the dorsal prefrontal region or are not associated with histological disruption as seen in routine myelin stains. Supported by MH60877, MH64168, and the Lieber Center for Schizophrenia Research at the Department of Psychiatry, College of Physicians and Surgeons of Columbia University.

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EXPRESSION LEVELS AND CELLULAR LOCALIZATION OF ERBB RECEPTORS mRNAs IN THE DORSOLATERAL PREFRONTAL CORTEX IN SCHIZOPHRENIA. Senda Beltaifa[1], Amanda J. Law[2], Thomas M. Hyde[1], Benjamin McClintock[1], Mary M. Herman[1]*, Paul J. Harrison[2], Joel E. Kleinman[1] and Cynthia Shannon. Weickert[1]. [1]CBDB, NIMH, NIH, Bethesda, MD. [2]Department of Psychiatry, University of Oxford, Oxford, UK.

Neuregulin-1 (NRG-1) is a neuronal growth factor and susceptibility gene for schizophrenia. In this study, we have measured NRG-1 receptor mRNAs (ErbB2, 3 and 4) in the DLPFC of schizophrenics and controls using in situ hybridization. ErbB2 and 4 mRNAs were predominantly expressed in the gray matter relative to white matter and did not differ in patients relative to controls ($p = 0.70$ for both). ErbB3 mRNA was expressed in both gray and white matter, with a particularly strong signal in white matter of some individuals. ErbB3 mRNA levels did not significantly differ in either gray or white matter when comparing normals versus schizophrenics. However, when ErbB3 mRNA levels were expressed as a ratio of gray matter to white matter, patients with schizophrenia had a significant decrease in the ratio ($t = -2.397$, $df = 23$, $p = 0.025$). This observation raised the question of the cellular source of ErbBs mRNA which was not readily determinable in these human specimens, so we addressed it in non human primate. In subcortical white matter, ErbB3 mRNA was localized to both glial cells and interstitial neurons; both neurons and glia of the gray matter expressed ErbB3 mRNA at low levels. ErbB2 and ErbB4 mRNAs were mainly expressed by neurons of the gray matter, with low expression in cells of the white matter. Our results suggest that there may be a maldistribution of ErbB3-expressing cells or an increase in ErbB3 expression per cell in the white matter of patients with schizophrenia.

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CONSISTENT LOSS OF SUBICULAR DENDRITIC SPINES IN SCHIZOPHRENIA AND MOOD DISORDERS. A.J. Dwork[1,2,3]*, Branislav Mancevski[1,2], Snežana Rauški[1], Tereza Serafimova[5], Natasa Davčeva[5], Matthew Kurzon[1], Aleksej Duma[2,5], J. John. Mann[1,2,4] and Gorazd Rosoklija[1,2,5]. [1]Department of Neuroscience, New York State Psychiatric Institute; and [2]Departments of Psychiatry, [3]Pathology, and [4]Radiology, College of Physicians and Surgeons of Columbia University, New York, NY. [5]Institute for Forensic Medicine, University "Ss. Cyril & Methodius," Skopje, R. Macedonia.

We reported a profound loss of dendritic spines in the subiculum in schizophrenia and mood disorders. Since the previously reported psychiatric cases were obtained from a different autopsy service than the nonpsychiatric cases, we sought to repeat the study in a series of cases from a single autopsy service. We also sought to use an improved Golgi technique, and to study a younger sample than previously, with less chronic illness and less extensive treatment. In this study, we used Golgi-Kopsch staining to quantify post mortem spine density in 13 mood disorder cases, 6 schizophrenia cases, and 5 nonpsychiatric cases, all autopsied at the Institute for Forensic Medicine, Skopje, R. Macedonia. Spine density on the main shaft of the apical dendrite of subicular pyramidal cells was 80% lower in both psychiatric groups than in the nonpsychiatric controls, with no overlap between controls and psychiatric cases. Spine loss was independent of duration of illness and unrelated to treatment, and was severe even in suicides with first episode, untreated depression. We conclude that the loss of spines is inherent to schizophrenia and mood disorders, at least when the clinical outcome is poor. This may be the most reproducible neuropathological pathological abnormality in serious psychiatric disorders. Support contributed by: National Institute of Mental Health grants MH64168, MH60877, and MH62185, grants from the National Alliance for Research on Schizophrenia and Depression and the American Foundation for Suicide Prevention, and by the Lieber Center for Schizophrenia Research in the Department of Psychiatry, Columbia University.

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HUMAN NEURAL STEM CELLS TRANSPLANTED INTO THE BRAIN OF RAT INTRACEREBRAL HEMORRHAGE STROKE MODEL PROMOTE FUNCTIONAL RECOVERY. S. U. Kim[1,4], K. S. Kim[1], H. J. Lee[1,2], E. J. Kim[1], D. H. Hwang[1], I. H. Park[1] and S. W. Jeong[3]. [1]Brain Disease Research Center, Ajou University School of Medicine, Suwon, Korea. [2]Department of Animal Science, Korea University, Seoul, Korea. [3]Department of Neurology, Ilsan Paik Hospital, Goyang, Korea. [4]Department of Neurology, University of British Columbia, Vancouver, Canada.

Human neural stem cells (NSCs) with self-renewal and multilineage differentiation properties would facilitate development of stem cell based cell therapy for human neurological disorders. We have isolated clonal human NSC lines that have been immortalized via a tetracycline (Tet) responsive *v-myc*; addition of Tet in the medium activates oncoprotein allowing the NSCs to proliferate rapidly, while in the absence of Tet NSCs differentiate into neurons. HB2.G2, one of the NSC cell lines, shows normal human karyotype of 46XX, doubling time of 36 hr and expresses nestin and ABCG2, cell type specific markers for NSCs. In the absence of Tet, >70% of G2 cells differentiate into neurons by expressing neuron specific markers (beta-tublin III, NF-L, NF-M, NF-H and MAP2), and sodium channels. Following transplantation in striata of rats with intracerebral hemorrhage (ICH) stroke, behavioral improvement was observed. Experimental ICH was induced by intrastriatal administration of collagenase in adult rat brain, and 3 days later G2 human NSCs or saline injected into strita. The animals were evaluated for 8 weeks using modified limb placing and rotarod tests. Grafted human NSCs migrated selectively to the peri-hematomal areas and differentiated mostly into neurons, and a marked behavioral improvement was demonstrated 2-8 weeks post-operation. A good survival and differentiation of grafted G2 human NSCs were demonstrated by immunohistochemistry with antibodies specific for neurofilament, MAP2 and GFAP. These results indicate that the immortalized human NSCs have great potential in clinical utility for cell therapy in human neurological disorders particularly in patients suffering with stroke.

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MOLECULAR MECHANISMS IN BLOOD-BRAIN BARRIER BREAKDOWN FOLLOWING BRAIN INJURY. Sukriti Nag[1], Roopa Venugopalan[1] and Duncan J. Stewart[2]. [1]Toronto Western Research Institute, University Health Network, University of Toronto, Toronto, ON, Canada. [2]Terrance Donnelly Heart Center, St. Michael's Hospital, University of Toronto, Toronto, ON, Canada.

The mechanism of early blood-brain barrier (BBB) breakdown whether by enhanced caveolae or breakdown of tight junctions remained controversial following the era of ultrastructural studies. In this study we localized caveolin-1, a major constituent of caveolae and the tight junction proteins–claudin-3, claudin-5, occludin and zonula occludens (ZO)-1 in control rats and in the rat cortical cold injury model at 6 hrs to 6 days following injury. Normal cerebral microvessels showed punctuate endothelial immunoreactivity for caveolin-1, while claudin-5 and occludin expression was observed as longitudinal strands in endothelium. During the first phase of BBB breakdown following injury at 12 hrs and 2 days, western blot analysis showed a 3.5 fold increase in caveolin-1 expression at the lesion site while a 3-fold and 1-fold decrease in expression of occludin and claudin-5 proteins respectively occurred only at days 2 and 4 post-injury. In order to determine if the vascular segments showing altered immunoreactivity for these proteins were also segments with BBB breakdown, dual labeling for these proteins and fibronectin was done. These results were analysed by laser scanning confocal microscopy which demonstrated that only lesion vessels with BBB breakdown to fibronectin showed increased caveolin-1 and loss of occludin and reduced localization of claudin-5. These studies support the hypothesis that cerebral endothelial cells initially respond to injury by enhanced caveolae which cause BBB breakdown. Persistence of the pathologic state results in structural damage to endothelial cells and breakdown of tight junctions and eventually the entire cell breaks down.

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LOW LEVELS OF HEXOKINASE II IN MALIGNANT GLIOMAS WITH 1P19Q DELETIONS. Mark T. Curtis*, J. Pastorino and D. Craig Hooper. Thomas Jefferson University Medical College, Philadelphia, PA.

Glioblastomas are resistant to chemotherapy whereas malignant gliomas with deletions of 1p19q respond to chemotherapy. Chemotherapeutic agents act through mitochondrial-dependent death pathways. Hexokinase II (HXK II) inhibits apoptosis and is elevated in malignant gliomas. Mitochondrial binding of HXK II is known to reduce sensitivity to chemotherapeutic agents in tumor cells. Thus, HXK II may be a critical tumor pro-survival factor in glioblastoma. Immunohistochemistry and real time quantitative PCR were utilized to compare HXK II mRNA and protein levels in glioblastomas with those of malignant gliomas with 1p19q deletions. Using real-time quantitative PCR we determined that mRNA levels for HXK II in glioblastomas were 10-fold greater than HXK II mRNA levels in the malignant gliomas with 1p19q deletions. HXK II mRNA levels were also measured in brain tumor cells selected using laser microdissection. The laser microdissected glioblastoma cells showed a 20-fold greater quantity of HXK II mRNA compared to the levels in tumor cells from a malignant glioma with 1p19q deletion. Immunohistochemistry was used to compare cellular HXK II protein levels. The immunohistochemical staining for HXK II in malignant gliomas with 1p19q deletion was markedly reduced (score 1.1 on a scale of 1-4) as compared to the cellular staining in glioblastomas (score 3.8 on a scale of 1-4). Our data demonstrate that HXK II mRNA and protein levels are markedly lower in malignant gliomas with deletions of 1p19q compared to glioblastomas. The different levels of HXK II may play a role in the sensitivity of brain tumors to chemotherapy.

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OLIGODENDROGLIAL NEOPLASMS WITH PSEUDOPALISADING NECROSIS: A CLINICOPATHOLOGIC AND FISH STUDY OF 44 CASES. C. Ryan Miller and Arie Perry*. Washington University School of Medicine, Division of Neuropathology, St. Louis, MO.

Oligodendrogliomas have characteristic whole arm chromosomal deletions of 1p and 19q, the presence of which has been correlated with both improved therapeutic responsiveness and overall survival (OS). Pseudopalisading necrosis (PN) is one of the defining features of glioblastoma, the WHO grade IV astrocytoma. However, PN is also rarely encountered in oligodendroglial tumors, where its prognostic and grading implications remain controversial. Therefore, we investigated 44 oligodendrogliomas and mixed oligoastrocytomas (MOAs) containing foci of both classic oligodendroglial cytology and PN, including 41 (93%) with fluorescence in-situ hybridization (FISH) data. Seventeen (39%) were "secondary", starting as grade II or III oligodendroglial tumors 1 to 16 (median 4.5) years prior. Pure oligodendrogliomas with PN showed a high rate of combined 1p and 19q deletions (14 of 15, 93%). In contrast, only 2 of 26 (8%) MOAs had codeletions, although solitary 19q deletions were detected in 13 (50%). Of 16 cases with sufficient followup, 8 (1 oligodendroglioma, 7 MOAs) demonstrated "GBM-like" behavior (OS 3-15 months) and 8 (3 oligodendrogliomas, 5 MOAs) had favorable outcomes (OS 3-9.8 years). No obvious morphologic or genetic differences were noted between these two groups. We conclude that oligodendrogliomas and MOAs with PN are genetically similar to their counterparts lacking this feature. Additional clinical followup and genetic studies for p16, PTEN, and EGFR status are in progress. Recommendations for the nomenclature of oligodendroglial tumors with PN will require additional studies, though these preliminary data suggest that at least two distinct biologic subsets may exist.

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QUANTITATIVE PROTEOMIC ANALYSIS OF OLIGODENDROGLIOMAS WITH AND WITHOUT 1P/19Q DELETION. Robert Rostomily, Donald Born, Ellsworth Alvord, Catherine Pan, Jinghua Jin, Thomas Montine and Jing Zhang. Departments of Neurosurgery and Pathology, University of Washington School of Medicine, Seattle, WA.

Approximately 50 to 70% of WHO grade II oligodendrogliomas demonstrate combined loss of chromosome 1p & 19q. Chromosome 1p/19q deletion, appearing early in tumorigenesis, is associated with improved clinical outcomes, including response to chemotherapy. Although many hypotheses have been proposed, the molecular mechanisms underlying improved clinical outcomes with 1p/19q deletion in oligodendrogliomas have not been characterized fully. We employed an unbiased proteomic approach, microcapillary liquid chromatography mass spectrometry (LC-MS) with isotope-coded affinity tags (ICAT), to quantify relative changes in the proteome of oligodendrogliomas, as determined by multiple independent neuropathologists, with and without 1p/19q deletion, following conventional biochemical separation of pooled tumor tissue into nuclei- mitochondria- and cytosol-enriched fractions. Preliminary results demonstrated that among >200 proteins identified in the cytosolic fraction, about 30 proteins changed significantly in their relative abundance (>100%) in morphological oligodendrogliomas with 1p/19q deletion as compared to those without 1p/19q deletion. Some of these proteins appear to be involved in signal transduction whereas others have been implicated in tumorigenesis. Currently, we are finalizing analysis of all cellular fractions and validating proteins related to 1p/19q deletion in individual oligodendrogliomas. It is anticipated that this type of analysis will allow us to generate a roster of proteins that may be developed into specific biomarker panels for predicting oligodendrogliomas with better prognosis and/or identifying new therapeutic targets for this glioma.

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GENETIC ANALYSIS OF CONGENITAL GLIOBLASTOMA. Amilcar A. Castellano-Sanchez[1], Bahig Shehata[1], Azita Djalilvand[1], Cynthia Hawkins[2], Robert B. Yost[3], Claudia Greco[4], Hiroko Ohgaki[5], Arie Perry[6] and Daniel J. Brat[1]*. [1]Emory University School of Medicine, Atlanta, GA. [2]The Hospital for Sick Children, Toronto, ON, Canada. [3]Children's Healthcare of Atlanta at Scottish-Rite, Atlanta, GA. [4]University of California, Davis, CA. [5]International Agency for Research on Cancer, Lyon, France. [6]Washington University School of Medicine, St. Louis, MO.

Glioblastoma (GBM) occurs rarely as a congenital neoplasm. We examined the genetic alterations of 6 congenital GBMs in 3 males and 3 females ranging in age from 6 days to 3 months (mean 26 days). They had classic features of GBM including diffuse infiltration, dense cellularity, high mitotic activity, endothelial proliferation and pseudopalisading necrosis. Our genetic analysis utilized paraffin-embedded tissue and included FISH analysis for EGFR, 9p21 (p16/CDKN2A) and 10q (PTEN/DMBT1) copy numbers; sequencing of the PTEN and TP53 genes; and immunohistochemistry for EGFR and p53. We uncovered 10q deletions in 2/6 cases by FISH. EGFR gene status was normal in 4/5 cases and associated with polysomy 7 in 1; no EGFR amplifications were noted. FISH analysis of 9p21 was normal in 4/5 cases tested and showed polysomy 9 in 1; no deletions were detected. No mutations of TP53 or PTEN were noted in any of the 5 cases tested. However, nuclear p53 immunoreactivity was strong and diffuse in 5/6 cases. Immunohistochemistry for EGFR showed no overexpression, with absent or mild staining in all 6 cases. In summary, although losses of chromosome 10 were noted in 2/6 cases, these 6 congenital GBMs lacked TP53 mutations, PTEN mutations, EGFR amplifications and p16/CDKN2A deletions. Nuclear overexpression of p53 was typically strong, however, indicating that other mechanisms could be relevant to p53 dysregulation. We conclude that despite histologic similarities, congenital GBMs are genetically distinct from their adult counterparts. Additional studies are needed to identify the specific tumorigenic alterations of congenital GBMs.

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PARTICULAR MICRORNAS (miRNAs) HELP TO DISCRIMINATE BRAIN TUMOR FROM NORMAL BRAIN TISSUE. Peter T. Nelson* and Zissimos Mourelatos. University of Pennsylvania, Philadelphia, PA.

miRNAs are the most abundant small (~22 nucleotides) regulatory RNA species in animals, constituting ~5% of the human transcriptome. miRNAs exert their influence by regulating translation of 'target' mRNAs. Up to 1/3rd of human mRNAs may be regulated thusly. Biologically active miRNAs are bound to a protein family called Argonaute. One problem with analyzing miRNA biology is the relative lack of tools that are specifically tailored to study these remarkable molecules. We have developed and applied new techniques and reagents to the study of miRNAs in the context of neuropathological specimens, including brain tumors. We studied 60 different brain tissues (~1/2 tumors) with the RNA-primed Array-based Klenow Enzyme (RAKE) platform, which is a unique microarray platform for high-throughput profiling of miRNAs, suitable for use in formalin-fixed paraffin-embedded (FFPE) tissue (see Nelson PT et al, Nature Methods 1:155-161). We found that the expression patterns of tumors clustered distinctly from normal tissue (including gliotic tissue). Furthermore, specific miRNAs could distinguish normal from neoplastic tissues, and adult from fetal brain. Another new tool that we developed is a monoclonal antibody against Argonaute proteins. We have applied our anti-human Argonaute antibody to FFPE brain tumor tissue, and preliminary evidence suggests that higher-grade glioma cells stain relatively intensely. Since the only known role of Argonaute proteins is to mediate the function of miRNAs, the apparent up-regulation of Argonaute proteins, in the context of an altered repertoire of miRNAs (as shown by the RAKE platform), may indicate an important role for miRNAs in brain tumor biology.

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EVALUATION OF RB GENE AND CYCLIN-DEPENDENT KINASE INHIBITORS P21 AND P27 IN PLEOMORPHIC XANTHOASTROCYTOMA. Elisabeth Rushing[1]*, Martha Quezado[2], Regia Evangelista[3] and Mariarita Santi[4]. [1]AFIP, Washington, D.C. [2]National Cancer Institute/NIH, Bethesda, MD. [3]BIOPSE Laboratories, Brazil. [4]Children's Hospital, Washington, D.C. *Sponsor.

Pleomorphic xanthoastrocytoma (PXA) is a rare, circumscribed astrocytic tumor that usually occurs in the superficial cerebral hemispheres in young adults and children. Most patients have a favorable prognosis, but recurrence and malignant transformation have been reported. In diffuse gliomas, approximately one-third demonstrate mutations of the Rb gene. Low expression level and high degradation activity of p27 are known to constitute independent prognostic factor in patients with malignant gliomas, while p21 expression have variable labeling ranges. The molecular and genetic basis for tumorigenesis and progression of PXA are still largely unknown, including the status of the Rb gene and of cyclin-dependent kinase inhibitors p21 and p27. In the present study, biopsy samples from 11 PXAs were examined immunohistochemically for RB, p21, and p27 expression. All PXAs exhibited nuclear Rb accumulation (many nuclei in 7 cases and few in 4), and nuclear expression of p21. Diffuse nuclear immunolocalization for p27 was seen in 8 cases, focally in 2, and was not present in the remainder case. The cases with focal and negative p27 nuclear expression had few Rb positive nuclei. The majority of PXAs appear to have preserved RB, p21, and p27 functions. Additional studies are necessary to investigate whether cases with focal or negative p27 expression coupled with few positive RB nuclei are associated with increased risk of recurrence or malignant transformation.

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EVALUATION OF NUCLEIC ACID INDEX IN BRAIN TUMORS. Martha Quezado[1]*, Elisabeth Rushing[2], Mariarita Santi[3], Stephen Wincovitch[1], Susan Garfield[1] and David Berman[1]. [1]National Cancer Institute/NIH, Bethesda, MD. [2]AFIP, Washington, D.C. [3]Children's Hospital, Washington, D.C. *Sponsor.

Nucleic acid derangements are the hallmark of many neoplastic processes, and can be detected earlier than histologic changes. The nucleic acid index (NAI) analyzes nucleic derangements in histologic sections at the level of the individual cell and its environment. One of the authors has previously demonstrated that lower NAI values in melanocytic lesions appear to correlate with malignant potential and inversely with mitotic index and Breslow thickness. We introduce NAI to analyze nucleic acid derangements in brain tumors with similar histology but different malignant potential: pleomorphic xanthoastrocytoma (PXA) and glioblastoma multiforme (GBM). Confocal laser scanning microscopy was performed on four pleomorphic PXA and two GBM cases, and one normal brain tissue stained with acridine orange (AO), a fluorescent stain for DNA and RNA. The NAI, calculated by measuring the fluorescence intensities of AO in nuclei relative to cytoplasm, reflects the concentration of DNA relative to RNA. Among the PXA group, NAI values were about 3 (2 cases), 2.5 (1 case) and 2 (1 case). The NAI values for the GBM were about 2.5, and 2.6, while the normal brain tissue was 6. Our preliminary results indicate that normal brain tissue and benign lesions tend to have higher NAI; an absolute correlation with malignancy, as noted in melanocytic lesions, is not always clear cut. NAI index is an objective, quantitative measure for nucleic acid derangements in brain tumors and may potentially be used to differentiate reactive from low grade neoplastic lesions. Additional studies are necessary to verify correlation with malignant potential.

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MICROARRAY-BASED IDENTIFICATION OF DIAGNOSTIC MARKERS FOR CHOROID PLEXUS TUMORS. Werner Paulus[1]*, Martin Hasselblatt[1], Christine Böhm[2], Vinzenz Dinh[1], Lars Tatenhorst[1], Shigehisa Hirose[3] and Christian H. Rickert[1]. [1]University Hospital, Münster, Germany. [2]Axaron Bioscience, Heidelberg, Germany. [3]Tokyo Institute of Technology, Tokyo, Japan.

The diagnosis of choroid plexus tumors and their differentiation from other papillary brain tumors and metastatic carcinomas may pose difficulties. In order to identify diagnostic markers, we compared DNA microarray based gene expression profiles of choroid plexus epithelia (n = 8) and ependymal cells (n = 6) micro-dissected from human autopsy brains as well as a surgical specimen of choroid plexus papilloma. Protein expression of choroid plexus genes was evaluated using tissue arrays representing normal choroid plexus, choroid plexus papilloma, and a panel of primary brain tumors and cerebral metastases. Forty-six genes were found to be overexpressed in choroid plexus epithelia (expression level more than seven-fold lower or absent in ependymal cells) and expressed in the papilloma. In addition to transthyretin, these included genes coding for ion channels, coagulation factors, enzymes, proteins involved in proliferation and development, receptors and several cytoskeletal proteins. Immunohistochemistry confirmed expression of inwardly rectifying potassium channel Kir7.1 in normal choroid plexus (34/35) and choroid plexus papilloma (12/19), but not in other primary brain tumors and cerebral metastases (0/102). Similarly, stanniocalcin-1 stained normal choroid plexus (32/36) and choroid plexus papilloma (16/19), while weak staining was seen in only 2/102 primary brain tumors and cerebral metastases. In addition, antibodies directed against coagulation factor V, glutathione peroxidase 3, pigment epithelium derived factor, serotonin receptor 5HTR2C, lumican and plastin-1 revealed varying degrees of specificity and sensitivity. We conclude that antibodies directed against Kir7.1, stanniocalcin-1 and other choroid plexus antigens might serve as valuable tools in the diagnosis of choroid plexus tumors.

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ABETA OLIGOMERS IN AGING AND ALZHEIMER DISEASE. Eileen H. Bigio[1]*, Mary P. Lambert[3], Pamela Shaw[2], Pascale N. Lacor[3], Kirsten L. Viola[3] and William L. Klein[3]. [1]Northwestern University Feinberg School of Medicine, Chicago IL. [2]Northwestern Alzheimer Disease Center, Chicago IL. [3]Northwestern University, Evanston IL.

Soluble Abeta oligomers are a significant toxic Abeta species hypothetically linked to synapse degeneration (Klein et al, TiNS, 2001), which for over a decade has been known as the best correlate of cognitive decline in AD. Using polyclonal Abeta oligomer antibody (M94), we recently showed that Abeta oligomers are strikingly elevated in AD brains (Gong et al, PNAS, 2003) and that oligomers obtained from AD brain bind specifically to synapses in hippocampal cultures (Lacor, et al. J. Neurosci, 2004). In the latter study we also saw M94 labeling of neuritic and diffuse plaques. In the current study, we used 20C2, a specific monoclonal Abeta oligomer antibody, to survey autopsy brains from subjects aged 53–107, comparing 20 with AD pathology ranging from CERAD A/Braak I to CERAD C/Braak VI to 9 with no AD pathology. Results show that 20C2 can be used successfully in human brain tissue sections and that it has a variety of labeling patterns. Labeling is confined to regions of the brain that develop AD pathology, and in these regions, some of the labeling co-localizes with senile plaques and some does not. 20C2 labeling increases with increasing AD pathology, and the labeling correlates with relative intensity of Abeta oligomers by dot-immunoblotting. More importantly, we demonstrate "peri-neuronal" labeling, which is likely due to the synaptic labeling shown previously in cell culture. Data are consistent with the hypothesis that synaptic targeting by oligomers occurs at the earliest stages of AD.

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DEGRADATION OF PERINEURONAL NET AROUND PARVALBUMIN-POSITIVE NEURONS IN ALZHEIMER'S DISEASE: POSSIBLE INVOLVEMENT OF MATRIX METALLOPROTEASES. Seth Love*, Shabnam Baig and Gordon K Wilcock. Department of Clinical Science at North Bristol, University of Bristol, Bristol, UK.

The perineuronal net (PN), a specialised region of extracellular matrix, is involved in regulating the neuronal microenvironment, particularly around GABAergic neurons containing the calcium-binding protein, parvalbumin. We have investigated the degradation of PN in Alzheimer's disease (AD), and the possible influence of parenchymal tau, Aβ, activated microglia, and the matrix metalloproteases MMP-3 and -9 on this process. Paraffin sections of frontal lobe from 100 cases of AD and 45 controls were labeled with *Wisteria floribunda* agglutinin for PN or immunostained for parvalbumin, tau, Aβ, MHC class II antigen, or MMP-3 or -9. Whereas the density of parvalbumin-positive neurons remained normal in AD, the density of neurons surrounded by PN was reduced by ~2/3 ($p < 0.001$). Combined fluorescent imaging revealed partial degradation of PN around some parvalbumin-positive neurons. The amounts of phosphorylated tau, Aβ and MHC class II antigen did not differ significantly in areas of cortex with and without intact PNs. Both MMP-3 and -9 were detectable perineuronally. The MMP-3 -1171 5A/6A promoter polymorphism was significantly associated with AD ($p = 0.015$) whereas the MMP-9 -1562 C/T polymorphism was not. In AD, parvalbumin-positive neurons tend to be spared but show degradation of the surrounding PN. This does not correlate topographically with neurofibrillary pathology, parenchymal Aβ or microglial activation but may be related to MMP activity.

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ACTIVATION OF NEUROGENESIS GENES AND SYNAPTIC TERMINAL REGENERATION IN AD. Cheryl Lin, Celia Williams, Li Zhou, Joshua Sonnen, Carol McCleary, Bryan M. Spann and Carol A. Miller*. Keck School of Medicine, University of Southern California, Los Angeles, CA.

Synaptic sprouting and plasticity occur throughout life even during the aging process. Synaptic terminals are affected early in AD pathogenesis. We predict that changes in expression of neuronal mRNAs, participating early in the disease, may include those also activated during neurogenesis. Frontal cortices were obtained post-mortem from clinically and cognitively characterized patients: 3 age-matched normal controls, 2 MCI and 4 early to severe AD patients. The MCI and AD patients showed a decline in parameters of executive function even prior to NFT histopathology. We examined doublecortin (DCX), Tuj1, and GAP43 for immunolocalization and by Western blots. Immunoperoxidase stains of frontal cortex revealed neuronal somata minimally positive for DCX in normal, age-matched controls but enhanced in AD in regions with Aβ deposition. On Western blots of lysates from highly enriched synaptoneurosome preparations, densitometric analysis revealed increased DCX and TUJ1 protein expression in AD compared to controls. mRNA isolated from synaptoneurosome preparations was analyzed using human Affymetrix HG-U133A microarrays. GAP43, which is locally translated in the axon and growth cone, and DCX each showed an increase in expression in early AD but were down-regulated in more advanced disease and thus are directly correlated with disease progression. These results confirm neurogenesis-related genes are active throughout life, and suggests they may be up-regulated in MCI patients. We conclude that in AD, attempts at synaptic regeneration are ongoing early in the disease but decline thereafter, perhaps indicating a switch in affected neurons to apoptotic pathways.

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ISOPEPTIDE CORRELATES WITH NEUROPSYCHOLOGICAL STATUS BETTER THAN TISSUE TRANSGLUTAMINASE PROTEIN OR ENZYME ACTIVITY LEVEL. Deng-shun Wang[1], David A. Bennett[2], Elliott J. Mufson[2] and Dennis W. Dickson[1]*. [1]Mayo Clinic, Jacksonville, FL. [2]Department of Neurological Sciences and Rush Alzheimer's Disease Center, Chicago, IL.

Background: Extensive protein crosslinking and aggregation are important molecular events in Alzheimer's disease (AD). Tissue transglutaminase (tTG) can catalyze crosslinking of tau and β-amyloid. **Objectives:** To measure tTG, tTG enzyme activity and isopeptide, which is the product of tTG, in brain and to relate them to cognitive measures. **Methods:** tTG and iso-peptide levels were measured in frontal gray matter of 10 normal (NCI), 10 mild cognitive impairment (MCI) and 9 AD brains from the Religious Orders Study using immunochemical methods. tTG enzymatic activity was measured with a fluorescence assay in the same samples. **Results:** tTG and isopeptide levels in total homogenates were significantly greater in AD than NCI. tTG and isopeptide levels in MCI were intermediate between AD and NCI, but not different from either. There was a trend for increased tTG activity in AD compared to MCI and NCI, with increased activity in MCI and AD of 28% and 37%, respectively. Only the difference between AD and NCI was significant. Isopeptide level in formic acid extracts was significantly greater in AD than both NCI and MCI. The levels of formic acid-extractable isopeptide were highly correlated with both Mini-Mental State Exam ($R = -0.680$) and global neuropsychologic z-scores ($R = -0.894$). **Conclusions:** The results indicate that accumulation of isopeptide, presumably due to tTG activity, may contribute to progressive cognitive decline in AD. The strongest correlations were for isopeptide in formic acid extracts, which are enriched in insoluble tau, suggesting that tau may be a substrate for tTG during pathogenesis of AD.

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NOTCH-1 CLEAVAGE IN PRION DISEASES CORRELATES WITH PERTERATIONS OF γ-SECRETASE COMPONENTS. Jared L. Clever, Nako Ishikura, Eric Huang and Stephen J. DeArmond*. University of California, San Francisco, CA.

Last year we showed that early-occurring dendritic atrophy in prion diseases is caused by cleavage of Notch-1 releasing its intracellular domain, designated NICD. NICD is a transcription factor that activates the Hes family of repressor genes known to inhibit expression of pro-neuronal genes that maintain dendrites during CNS development. Western blot analysis of prion-infected mouse cortex revealed a 2-fold increase in Hes5 expression. IHC verified that result by showing a marked increase in Hes5 in prion infected neuronal nuclei and cytoplasm. We also tested whether increased NICD is the result of PrP^{Sc}-induced increase of γ-secretase activity. γ-secretase is a multi-protein complex that includes both Nicastrin and Presenilin-1. Western blot analysis showed an ~2-fold increase in cerebral cortical Nicastrin during mouse scrapie and in scrapie-infected ScGT1 cells. The increase in cultured cells was due almost entirely to immature Nicastrin. Presenilin-1 showed only a modest increase. Both increased NICD and increased concentrations of components of γ-secretase support the hypothesis that increased γ-secretase activity occurs in prion diseases. It is reported that γ-secretase cleavage of Notch-1 occurs in lipid-raft domains, which is where PrP^{Sc} accumulates. That plus the results presented here argue that PrP^{Sc} accumulation may directly increase the rate of γ-secretase activity. Our results also argue that synaptic degeneration in prion diseases is a gene regulated process like apoptosis and raise the possibility that γ-secretase inhibitors might prevent the early synaptic degeneration that characterizes both prion diseases and Alzheimer's disease.

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SINGLE MOLECULE ANALYSIS OF PROTEIN AGGREGATION AND PRIONS BY SIFT. Hans A. Kretzschmar[1]*, Uwe Bertsch[1], Konstanze F. Winklhofer[2], Thomas Hirschberger[3], Jan Bieschke[1], Petra Weber[1], F. Ulrich Hartl[2], Paul Tavan[3], Jörg Tatzelt[2] and Armin Giese[1]. [1]Institute of Neuropathology, University of Munich, Munich, Germany. [2]Department of Cellular Biochemistry, Max-Planck-Institute for Biochemistry, Martinsried, Germany. [3]BioMolecular Optics, University of Munich, Munich, Germany.

Protein aggregation is a key event in a number of diseases such as Alzheimer's disease, Parkinson's disease and prion diseases. Dual-color scanning for intensely fluorescent targets (SIFT), which has been used in the diagnosis of prion diseaes, is presented here as a general technique to quantify and characterize protein aggregates. In the case of prion diseases the prion protein (PrPC), a neuronal glycoprotein, undergoes a conformational change from the normal, mainly alpha-helical conformation to a disease-associated, mainly beta-sheeted scrapie-isoform (PrPSc), which forms amyloid aggregates. This conversion depends on direct PrPC/PrPSc interaction. We have developed a high-throughput SIFT assay for the identification of drugs, which interfere with this interaction at the molecular level. Screening a library of 10,000 drug-like compounds yielded 256 primary hits, 80 of which were confirmed by dose-response curves. Among these, six compounds displayed an inhibitory effect on PrPSc propagation in scrapie-infected N2a cells. Four of these candidate drugs share an Ń-benzylidene-benzohydrazide (NBB) core microstructure. Preliminary experiments with NBB in scrapie-infected mice have shown positive effects. Thus the combination of a high-throughput *in-vitro* assay with the established cell culture system provides a rapid and efficient method to identify new anti-prion drugs, which corroborates that interaction of PrPC and PrPSc is a crucial molecular step in the propagation of prions. In addition, SIFT-based screening may facilitate the search for drugs against other diseases linked to protein aggregation.

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KINASE REGULATION OF AUTOPHAGY DURING MPP+ TOXICITY. Charleen T. Chu[1]*, Yasuo Uchiyama[2] and Jian-Hui Zhu[1]. [1]University of Pittsburgh School of Medicine, Pittsburgh, PA. [2]Osaka University, Osaka, Japan.

Parkinson's disease is a debilitating neurodegenerative movement disorder that may share pathogenic mechanisms with diffuse Lewy body disease, the second most common form of dementia. 1-Methyl-4-phenylpyridinium (MPP+) is a mitochondrial complex I inhibitor that preferentially injures dopaminergic neurons. While MPP+/MPTP have been used extensively to model parkinsonian neurodegeneration, specific inuury mechanism(s) remain to be elucidated. We found that MPP+ elicited an autophagosome-rich form of regulated cell death in SH-SY5Y cells. Caspase inhibitors did not confer protection from toxicity. In contrast, inhibitors of the extracellular signal-regulated protein kinase (ERK) pathway inhibited autophagy and conferred protection from MPP+ toxicity. Neither inhibition of phosphoinositide 3-kinase (PI-3K) nor siRNA knockdown of beclin 1 blocked MPP+-elicited autophagy, suggesting that regulation of pathologic autophagy in this system may differ from that of starvation or trophic deprivation systems. The role of autophagy in neuronal cell death is currently unknown. However, phospho-ERK is found in autophagocytosed mitochondria in degenerating human Parkinson's disease neurons. Taken together, these studies suggest that the altered subcellular compartmentalization of activated ERK observed in Parkinson's disease may serve to regulate mitochondrial autophagy during dopaminergic cell death. Supported by the National Institutes of Health (R01 NS40817).

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AMINO-TERMINALLY TRUNCATED Aβ PEPTIDE SPECIES ARE THE MAIN CONSTITUENT OF COTTON WOOL PLAQUES. Leticia Miravalle[1], Miguel Calero[2], Masaki Takao[1], Bernardino Ghetti[1] and Ruben Vidal[1]*. [1]Indiana Alzheimer Disease Center, Indiana University, Indianapolis, IN. [2]Centro Nacional de Microbiología, Majadahonda, Madrid, Spain.

Cotton wool plaques (CWPs) are round, mildly fluorescent, eosinophilic plaques that lack a central amyloid core and are immunopositive for antibodies against the amyloid-β (Aβ) peptide. CWPs have been reported in association with several mutations in the *Presenilin 1* (*PSEN1*) gene. The purpose of this study is to biochemically characterize the Aβ peptide species present in CWPs in association with the *PSEN1-V261I* and *PSEN1-V261F* mutations. Aβ peptides were purified from the cerebral and cerebellar cortices from one individual with the *PSEN1-V261I* mutation. CWPs were obtained by laser microdissection (LMD) from the cerebral cortex of one individual with the *PSEN1-V261I* mutation and one individual with the *PSEN1-V261F* mutation. The isolated Aβ peptide species were identified by western blot and MALDI-TOF mass spectrometry. In the cerebral and cerebellar cortices, the main Aβ peptide species detected were amino-terminally truncated Aβ peptides starting at residues Glu-3 and Glu-11 and ending at residues 42 and 43. Full-length Aβ1-42 and Aβ1-43 were underrepresented and Aβ1-40 was not detected. AβN11pE-42 and Aβ11-42 were the main constituents of the CWPs isolated by LMD. Other Aβ peptide species included AβN11pE-43 and Aβ11-43, AβN3pE-42 and Aβ3-42. This data indicates that amino-terminally truncated Aβ peptides may be of major importance in the pathological process of Alzheimer disease. Since CWPs in the cerebral cortex and diffuse amyloid plaques in the cerebellar cortex have a similar array of Aβ peptides, we hypothesize that tissue-related factors may play an important role determining the morphology of the Aβ deposits. Supported by Alzheimer's Association, P30AG10133.

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PROTEIN KINASE Mzeta COAGGREATES WITH GLUTAMATE AMPA RECEPTORS IN PERISOMATIC GRANULES IN ALZHEIMER'S DISEASE. Charles Y. Shao, John F. Crary, Todd C. Sacktor, William Oxberry and Suzanne S. Mirra. SUNY Downstate Medical Center, NY.

Alteration of synaptic plasticity is believed responsible for memory loss in Alzheimer's disease (AD). Molecules involved in synaptic plasticity, therefore, may play important roles in AD pathogenesis. In this regard, we have investigated the relationship between glutamate AMPA receptor (AMPAR) subunits and protein kinase Mzeta (PKMζ) in AD. AMPAR is well known for its crucial role in modulating NMDA receptor activity during long-term potentiation (LTP) and in other models of learning and memory. PKMz, the constitutively active, independent catalytic domain of atypical PKCζ, has been shown to be necessary and sufficient in maintaining LTP and enhancing conditioned memory in *Drosophila*. During LTP, PKMζ directly enhances glutamate AMPAR currents in postsynaptic neurons. Using antibodies specific to PKMζ and AMPAR subunits GluR1 and GluR2, we found that PKMζ associated with GluR1/2 in so-called perisomatic granules surrounding hippocampal pyramidal neurons. This association exhibited variable patterns. In some instances, PKMζ and GluR1/2 granules were closely approximated; in others, one or more PKMζ-positive fine granules existed inside GluR1/2-positive perisomatic granules. In some dystrophic neurites, PKMζ and GluR1/2 exhibited a striped pattern. Experiments using mouse brain tissue demonstrated a similar association between PKMζ and GluR1. Electron microscopy revealed that both PKMζ and GluR1 are predominantly localized at postsynaptic elements. Taken together, our data suggest that PKMζ dynamically interacts with AMPAR during normal synaptic function and that these interactions may lead to their co-aggregation into perisomatic granules in AD. This study was supported by NIH grant K07 AG 00959 to SSM.

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ALZHEIMER AND PARKINSON CHANGE IN HUMAN AGING BRAIN. Yuko Saito[1]*, Hiroshi Yamanouchi[2], Tomio Arai[2], Motoji Sawabe[2] and Shigeo Murayama[1]. [1]Tokyo Metropolitan Institute of Gerontology; and [2]Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan.

Objective: To clarify the relationship between Alzheimer disease (AD), Parkinson disease (PD) and Dementia with Lewy body (DLB). **Methods:** Serial 1395 autopsy cases from an aging cohort were examined. The mean age was 80.6 with male to female ratio being 758: 637. Parkinsonism and dementia were retrieved from the clinical records. The diagnosis of AD is based on dementia plus Braak's neurofibrillary tangle (NFT) stages equal to or above IV and senile plaque (SP) Stage C. Lewy body dementia (LBD) is defined as dementia accompanying Lewy body-related neuronal degeneration involving the central and peripheral autonomic, the nigro-striatal and the limbic/neocortical systems. Each case was classified into AD+LBD-, AD+LBD+, AD-LBD+ and AD-LBD-. Apolipoprotein E (Apo E) genotyping was examined in 1,114 of the 1,395 cases. **Results:** 120 cases (8.6%) were categorized into AD+LBD-, 45 (3.2%) AD-LBD+ and 16 (1.1%) AD+LBD+. The series included 28 (2.0%) PD cases, 16 (1.1%) of which were presented with dementia, with one AD+LBD+ and 15 AD-LBD+ cases. The male to female ratio in AD+LBD-, AD-LBD+ and AD+LBD+ was 0.53, 1.1 and 0.78, respectively. AD-LBD+ with SP Stage C but NFT Stage 0-III covers 29% of all AD-LBD+, higher than 11% of AD-LBD-. The apoE ε4 frequency in AD+LBD-, AD+LBD+, AD-LBD+ and AD-LBD- was 25%, 27%, 15% and 9.3%, respectively. **Discussion and Conclusions:** We adopted this approach to avoid confusion in the naming of AD, DLB and PD plus dementia. Our data confirms a mutual aggravating effect of AD and PD changes in the aging human brain.

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Aß42 SUPPRESSES THE ANTI-INFLAMMATORY ALTERNATIVE ACTIVATION STATE IN MICROGLIA: A POTENTIAL ROLE FOR ALZHEIMER'S DISEASE. Ryan T. Mott, Christine M. Hulette*, Michael P. Vitek and Carol A. Colton. Duke University Medical Center, Durham, NC.

Microglia are believed to play a pathologic role in Alzheimer's disease (AD) by entering a pro-inflammatory activation state under the influence of the 42 amino acid ß-amyloid (Aß42) peptide. Unfortunately, the role of Aß42 in the regulation of microglial activation has yet to be fully explored, especially in light of recent studies showing an anti-inflammatory alternative activation state in peripheral macrophages. Our objective was to characterize classical and alternative activation in microglia and to determine the effects of Aß42 on these activation states. Thus, we stimulated murine BV2 microglia with classical (IFN-γ) and alternative (IL-4) immune stimuli and assayed for gene expression (Real Time PCR) and nitric oxide (NO) production (Griess reaction). We selected a profile of six genes that are important for macrophage function, including inducible nitric oxide synthase (NOS2), TNF-α, arginase 1 (ARG1), mannose receptor (MR), found in inflammatory zone 1 (FIZZ1), and chitinase 3-like 3 (YM1). We show that classically activated microglia upregulate NOS2, TNF-α, and NO production, while alternatively activated microglia upregulate ARG1, MR, FIZZ1, and YM1. Moreover, we demonstrate that 1 μM Aß42 has no effect on resting or classically activated microglia, but does alter the ability of microglia to become alternatively activated by increasing ARG1 and decreasing MR, FIZZ1, and YM1 expression. We also examined a mouse model of AD (Tg-SwDI) and found that TNF-α is increased and FIZZ1 decreased in forebrain extracts. We conclude that Aß42 may function pathologically by suppressing microglial alternative activation.

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X-RAY FLUORESCENCE SPECTROSCOPY IN SITU QUANTIFICATION OF METALS/ELEMENTS IN ALZHEIMER DISEASE. Eileen H. Bigio[1]*, Tatjana Paunesku[1], Manjari Mishra[1,2], Stefan Vogt[3], Barry Lai[3], Jorg Maser[3] and Gayle E. Woloschak[1]. [1]Northwestern University Feinberg School of Medicine, Chicago, IL. [2]Northwestern Alzheimer Disease Center, Chicago, IL. [3]Argonne National Laboratory, Argonne, IL.

Metals and other elements, essential for biologic reactions, are also found in insoluble deposits in neurodegenerative disorders. Three reports demonstrate the use of x-ray fluorescence spectroscopy (XRF) in neurodegenerative disorders, but do not describe metals/elements within specific pathologic features of the disease. We performed XRF on 2 each severe AD and age-matched control cases to determine whether we could identify NFTs and neuritic plaques on XRF images, whether metal/element deposits were associated with these structures, and whether quantities of metals/elements in these tissue sections differed between AD and controls. We found overall differences between AD and controls in gray matter metals/elements. Using Gallyas stains of sections post-XRF, we identified probable NFTs and amyloid deposits, and more carefully analyzed metal quantities in these structures. We found high levels of Cu, Zn, & Fe in potential NFTs and high levels of Zn and Fe in potential amyloid deposits. We conclude that 1) x-ray fluorescence demonstrates metals/elements in human postmortem brain paraffin sections, 2) these concentrations differ between AD and control, 3) current XRF methods are able to resolve some subcellular structures (e.g., nuclei), to localize increased quantities of metals/elements in the cells, to target NFTs and amyloid deposits in AD, and to quantify metals/elements in NFTs and amyloid deposits. Lastly, XRF is a potentially powerful tool for further investigations into the mechanisms underlying the pathogenesis of AD and other neurodegenerative disorders.

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OREXIN-CONTAINING NEURONS AND SLEEP-WAKE DISTURBANCES IN ALZHEIMER'S DISEASE. E. G. Stopa[1], S. Soscia[1], R. Tavares[1], A. McGee[2] and D. G. Harper[3]. [1]Department of Pathology, Neuropathology Division, Brown Medical School, Providence, RI. [2]Deparment of Pathology, Boston University School of Medicine, Boston, MA. [3]Department of Psychiatry, McLean Hospital, Harvard Medical School, Belmont, MA.

Patients with Alzheimer's disease (AD) typically exhibit erratic patterns of sleep and wakefulness that may involve disruption of circadian- and sleep-related systems. Orexin/hypocretin depletion has been observed in the clinical syndrome of human narcolepsy as well as in narcoleptic animal models, and it may also mediate a circadian alerting signal. We propose that hypothalamic orexin/hypocretin deficiency may be a critical factor mediating the disturbances of sleep and wakefulness in Alzheimer's disease and related dementias. The distribution pattern of orexin/hypocretin immunoreactivity was examined in postmortem hypothalami of Alzheimer patients and compared to elderly controls to establish a putative role for orexin in the sleep-wake disturbances of patients with dementia. Orexin/hypocretin containing neurons and accompanying fibers were primarily found in the anterior olfactory region, infundibular region (arcuate nucleus), and lateral (perifornical) and supramamillary region of the posterior hypothalamus. Terminal fields were observed in the basal forebrain, particularly the nucleus basalis of Meynart, the arcuate nucleus and the perifornical region of the lateral hypothalamus. Qualitative analyses suggested a loss of orexin immunoreactivity in Alzheimer patients. ELISA analyses performed on hypothalamic tissue from AD patients and elderly controls yielded orexin concentrations that varied from 22 to 53 ng/mg total protein. Clarification of the impact of Alzheimer's and other dementias on orexin/hypocretin system could elucidate the etiology of circadian-linked and other behavioral disturbances seen in these patients and provide clues to possible treatment strategies. Support Contributed By: R01-AG20654; P30 AG13846; NIRG 2635 (Alzheimer's Association), and the Dept. of Veterans' Affairs.

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FACTORS INFLUENCING POST-MORTEM RNA INTEGRITY IN HUMAN BRAIN. C. R. Vanderburg[1], R. Pfannl[2], D. Tian[2], T.-R Kiehl[2], T. Hsi[1], E. T. Hedley-Whyte[2] and M. P. Frosch*[2,3]. [1]Harvard Center for Neurodegeneration and Repair; [2]Massachusetts General Hospital; and [3]Harvard Medical School, Boston, MA.

Many molecular and biochemical approaches to the study of neurologic disease depend on recovery of mRNA from post mortem brain tissue. Methods of estimating RNA quality include indirect measures such as perimortem circumstances, cause of death, post-mortem interval (PMI), tissue pH or time consuming direct observations using Northern blots or in situ hybridization. We measured RNA quality from 277 samples from 120 brains harvested for research (Vonsattel et al, 1995) in order to clarify factors that influence RNA preservation. Total RNA was extracted from fresh and archived frozen brain tissue using standard commercial reagents, and assayed with an Agilent Bioanalyzer. RNA quality was classified on a five point rating scale, based on the 18S and 28S peak shapes. One third of samples, coming from 44% of brains, had useful RNA preservation based on their scores, subsequently validated through qPCR. RNA quality did not correlate with clinical diagnosis, PMI, age, sex, and archived time (years). In addition RNA integrity varied in different structures within the same brain; therefore, each brain may have its own spatial pattern of RNA integrity. Our results suggest that 1) none of the clinical data predicted RNA integrity; 2) RNA integrity varies across anatomic areas; 3) with this technology, direct observation of RNA quality can be performed quickly (isolation requires 1 hour; assay, 30 minutes) and the derived information increases the utility of samples provided by a brain bank. (Supported by the Harvard Center for Neurodegeneration and Repair (HCNR); NIH grants AG05134 and NS038372).

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ALZHEIMER'S NEUROPATHOLOGY IN AFRICAN-AMERICANS AND WHITES. Miguel A. Riudavets[1,2], Ana Rubio[3], David Fowler[3], Christopher Cox[4], Gay Rudow[1] and Juan C. Troncoso[1]. [1]Johns Hopkins University School of Medicine, Baltimore, MD. [2]Armed Forces Institute of Pathology, Washington, D.C. [3]Office of the Chief Medical Examiner, State of Maryland, Baltimore, MD. [4]Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD.

To compare the frequency and severity of Alzheimer's lesions, i.e. Aβ deposits (plaques) and neurofibrillary tangles (NFT) in African-Americans (AA) and whites (W), we examined prospectively the brains of 78 males (34 AA and 44 W, 65-95 years of age) from consecutive autopsies conducted at the Office of the Chief Medical Examiner of Maryland between 2002 and 2004. Cases were selected independently of history of cognitive decline or dementia. Sections of the hippocampus, and entorhinal, middle temporal, middle frontal and occipital cortices were immunostained for Aβ and Tau. All cases were genotyped for ApoE. Plaques were present in 61.76% of AA and 54.55% of whites, and their severity was rated according to CERAD. NFT were present in 94.12% of AA and in 83.36% of W. The severity of NFT was rated into four groups corresponding to Braak scores: 0, Transentorhinal, Limbic, and Isocortical. For the Aβ variable, when ApoE is included in the analysis, neither race nor age is a significant independent variable, but ApoE4 ($p = 0.0003$) is a highly significant predictor. For the NFT variable, neither race nor ApoE is a significant independent variable, but age ($p = 0.0005$) is a highly significant predictor. ApoE4, appeared as a highly significant predictor of plaque severity both for AA ($p = 0.01$) and W ($p = 0.013$). In conclusion, in this series, race was not a significant factor in the development of Alzheimer's lesions, as suggested by some clinical studies. However, ApoE4 appears as a significant risk factor for the development of Aβ plaques in both races.

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NUCLEOCYTOPLASMIC TRANSPORT IN ALZHEIMER'S DISEASE: A STUDY OF KARYOPHERINα AND IMPORTINß1 IN RAT BRAIN. Lynette G. Sheffield and Suzanne S. Mirra*. SUNY Downstate Medical Center, Brooklyn, NY.

Ultrastructural studies of Alzheimer's disease (AD) have demonstrated a close relationship between nuclear pores and paired helical filaments of neurofibrillary tangles, nuclear irregularity in many tangle-bearing neurons, and nuclear pore aggregation in nearby neurons. These observations prompted our examination of the nuclear pore complex (NPC) and other proteins critical to nucleocytoplasmic transport. Our previous immunohistochemical study demonstrated NPC proteins at the nuclear envelope of all cells, highlighting nuclear irregularity in neurons in AD. Cytoplasmic accumulation of nuclear transport factor 2, a protein that transports cargo from the cytoplasm into the nucleus, also was observed in a subset of hippocampal neurons with and without tangles in AD but not controls. To continue our investigation of nucleocytoplasmic transport-related proteins, we labeled normal rat brain sections using antibodies directed against two proteins integral to a major nuclear import pathway: karyopherinα (NPI-1; Zymed) and importinß1 (NTFp97; Affinity BioReagents). Focal karypherinα labeling occurred in gray matter adjacent to the corpus callosum, filling the cytoplasm of neurons and extending into apical dendrites. In contrast, importinß1 had a more widespread distribution in neurons with a punctate pattern within the nucleus and nuclear envelope; no cytoplasmic label was observed. To our knowledge, this study represents the first investigation of karyopherin α and importinß1 in brain tissue.

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ALTERATIONS IN NEURONAL CALICPRESSIN PROTEIN EXPRESSION IN AGING AND ALZHEIMER'S DISEASE. John M. Lee*, Henry G. Brown, Susan O. McGuire and Casey N. Cook. Loyola University Medical Center, Maywood, IL

The activity of protein phosphatase 2B (calcineurin) has been shown to be decreased in Alzheimer's disease (AD), which may represent a possible mechanism(s) for the hyperphosphorylation of tau and subsequent neurofibrillary tangle (NFT) formation characteristic of the disease. Recently, mRNA expression of DSCR1 (Down's Syndrome Critical Region Gene 1), which encodes the protein calcipressin (identified as an endogenous inhibitor of calcineurin), was found to be upregulated in both Down's Syndrome (DS) and AD. Calcipressin is induced by oxidative stress and A *in vitro*, suggesting a link between the regulation of this protein and the pathology of both AD and DS. Using immunohistochemistry techniques (optical density), calcipressin protein expression in the temporal lobe (pyramidal neurons of cortical cell layers III and V) was shown to increase with aging ($r = 0.7522$; $p = 0.0313$), and also in mid to late stage AD compared to age-matched control patients ($t = 3.872$; $p = 0.0017$). In addition, there is a 88% increase in nuclear calcipressin immunoreactivity in AD ($p = 0.0001$), though cytosolic calcipressin levels are only increased by 20% ($p = 0.014$). This suggests a possible translocation of calcipressin from the cytosolic to nuclear compartment in the disease state. We also found that there was a positive correlation between total calcipressin positive pyramidal neurons and the number of NFT's. Interestingly, there appears to be a decreased nuclear staining in cells that contain NFT's. These data suggest that altered cellular regulation and trafficking of calcipressin may lead to decreased calcineurin activity and NFT formation.

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UNUSUAL FINDING OF BRAIN CRYSTALS IN A MULTIPLE SCLEROSIS PATIENT: A CASE REPORT. Vassil Kaimaktchiev[1], Theodore J. Lowenkopf[1,2], Oisin R. O'Neill[2], Kay L. Larkin[1] and Randal R. Nixon[1]*. [1]Oregon Health & Science University, Portland, OR. [2]Providence St. Vincent Medical Center, Portland, OR.

The patient was a previously healthy 30-year-old woman presenting with aphasia, dysarthria, right facial droop and transient arm weakness. CT founded multiple bilateral cerebral lesions. A MRI showed hemispheric and cerebellar enhancing lesions. The radiographic and clinical differential included lymphoma, glioma, MS, and viral encephalitis. Her family history was non-contributory. She was admitted and treated with dexamethasone. Further work-up included a brain biopsy and repeat imaging. Urinalysis performed acutely and at eight months revealed calcium oxalate crystals and increased levels of ethanolamine, alanine, and taurine, but otherwise normal renal function. Lumbar puncture was unremarkable; oligoclonal bands were negative. The cortical biopsy showed abundant achromatic crystals that colocalized with foamy macrophages and chronic inflammatory cells in greatest abundance in the perivascular spaces. There was moderate astrogliosis and focal mild demyelination. No atypical forms were identified, and there was no evidence of an infectious, neoplastic or ischemic process. The composition of the brain crystals remains unknown. The patient has made an excellent recovery and serial MRIs show regression of the inflammatory component. The findings are felt to be most consistent with MS suggesting the crystals are epiphenomena. Alternatively, the urinalysis findings suggest the possibility of oxalate. Cerebral oxalate crystals are extremely rare and usually associated with primary hyperoxaluria.

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CAUSES OF UNEXPECTED DEATH IN PATIENTS WITH MULTIPLE SCLEROSIS: A FORENSIC STUDY OF 50 CASES. Miguel A. Riudavets*[1,2], Ana Rubio[3], David Fowler[3], Carlos Pardo[1] and Juan C. Troncoso[1]. [1]Johns Hopkins University School of Medicine, Baltimore, MD. [2]Armed Forces Institute of Pathology, Washington, D.C. [3]Office of the Chief Medical Examiner of the State of Maryland (OCME), Baltimore, MD.

To determine the cause of death in patients with multiple sclerosis (MS) by examining autopsies of 50 subjects with MS from the OCME of Maryland. Between 1982 and 2004, the causes of death in 32 females and 18 males (mean age 45.8 years; range 25-69 years) were classified into: A) neurological complication directly related to MS; B) non-neurological complications or other medical causes; and C) accidents, etc. Of the 50 cases, in 43 there was a history of MS, but in 7 there was not, and the diagnosis was established by neuropathologic examination. Group A, 21 (42%) cases were directly related to a neurological complication; Group B, 14 (28%) cases were related to the following non-neurological and medical causes: ACVD 9 (18%), metabolic disorder 1 (2%), pulmonary embolism 3 (6%), and bronchopneumonia 1 (2%); Group C, 15 (30%) cases with the following causes: trauma 9 (18%), intoxication 5 (10%), and thermal injury 1 (2%). Thus, in the 50 subjects, death occurred naturally in 26 and from accidents, homicides, suicides or undetermined causes in 24. The majority of cases showed either chronic inactive (66.7%) or chronic active (15.6%) demyelinating lesions, mainly in the cerebral hemispheres. In some cases, it appears that demyelinating lesions, involving brain regions regulatory of cardiorespiratory activity, could be considered as the immediate cause of death, but a large proportion appears to be due to other causes such as accidents and trauma. Thus, it seems likely that taking specific precautions could prevent some deaths in MS.

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SOLITARY JUVENILE XANTHOGRANULOMA IN THE MIDBRAIN–REPORT OF A CASE. Chimène Kesserwan[1], Cheng Z. Liu[1], Paul C. Francel[2] and Kar-Ming Fung[1]. [1]Department of Pathology, University of Oklahoma Health Science Center, Oklahoma City, OK. [2]TPG Oklahoma Brain and Spine Institute, Edmond, OK.

An eight-year-old boy presented with nausea, vomiting, and tremor. Magnetic resonance imaging revealed a well-defined, solitary, enhancing mass in the midbrain with features suggestive of a thrombosed cavernous hemangioma. Physical examination did not reveal any lesion on the skin. Additional imaging studies and bone survey did not demonstrate any lesion in other locations. The excised specimen appeared as a small, oval, rubbery nodule. Histologically, the lesion was composed of a core of foamy histiocytes admixed with collections of chronic inflammatory cells, Touton giant cells, and collagen fibers. A substantial amount of hemosiderin depositions was also present. The xanthomatous core was surrounded by a shell of dense reactive gliosis that contain numerous Rosenthal fibers. On immunohistochemistry, the foamy histiocytes were strongly positive for CD68 but negative for S100 and CD1a. A diagnosis of juvenile xanthogranuloma was made. Juvenile xanthogranuloma occurs most commonly as solitary cutaneous lesion. Multiple lesions are seen in about one-tenth of the cases. Although systemic involvement is uncommon, there are well documented cases. Isolated involvement of the central nervous as demonstrated in this case is rare. On frozen sections, these lesions can mimic desmoplastic glial neoplasms because of the substantial gliosis and collagen fibers. On paraffin sections, these lesions must be distinguished from Langerhans' cell histiocytosis and infections.

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NEUROPATHOLOGY OF CORNELIA DE LANGE SYNDROME; A CASE REPORT. Irina Mikolaenko[1], Brad Randall[2] and Hannah Kinney[1]*. [1]Children's Hospital, Boston, MA. [2]University of South Dakota School of Medicine, Sioux Falls, SD.

Cornelia de Lange syndrome (CDLS) is a rare genetic syndrome characterized by a distinctive facial appearance, growth deficiency, and systemic malformations. Despite the fact that psychomotor and mental retardation are the most consistent features of this syndrome; very few reports of the neuropathological analysis of this syndrome exist. The aim of our study is to present a complete neuropathological analysis of an adult case of CDLS. The patient was a 41-year-old Caucasian woman with the clinical diagnosis of CDLS who was found apneic in bed in her group home. We received the formalin-fixed brain in consultation for detailed neuropathological examination. The major macroscopic findings were mild microcephaly (brain weight 1010g, expected weight 1050-1550 (average 1275) in grams), loss of volume of white matter, and anomalies of the temporal lobes bilaterally. Medial temporal lobes were excessively prominent compared to the normal control brain. Gyral pattern of the left temporal lobe was abnormal. Microscopically, we identified fusion of multiple gyri in the frontal and temporal lobes, hyperconvoluted inferior olives and dentate nuclei; and remodeling of the tectum of the brain stem with multiple dilated vascular channels surrounded by disorganized white matter bundles. Recent studies have shown that CDLS is dominantly inherited and caused by mutation in the human homolog of Drosophila melanogaster Nipped-B (NIPBL), which facilitates enhancer-promoter communication and regulates Notch signaling. The relationship of this mutation to the observed brain anomalies is not known and needs to be investigated.

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ENCEPHALOCELE WITH GIANT CELL FIBROBLASTOMA LIKE LESION. Veena Rajaram*, Tord Alden and Pauline M. Chou. Children's Memorial Hospital/Northwestern University, Chicago, IL.

Encephaloceles are neural tube defects with a prevalence of 0.5-2 per 1000 and a female preponderance. We identified a case of encephalocele with adjacent giant cell fibroblastoma-like changes. There is a single case report by Hirokawa M et al., of a giant cell fibroblastoma-like area in association with an encephalocele. Giant cell fibroblastoma (GCF) are soft tissue tumors frequently seen in association with dermatofibrosarcoma protuberance. There is some debate that GCF may represent reactive changes to the adjacent tumor. These tumors are positive for CD34 and vimentin. The single reported case of GCF like changes in association with encephalocele was negative for CD34. Of the 16 cases of encephalocele resected in our institute in the past 10 years, we identified 10 cases of encephalocele with GCF like changes. Histologically, these lesions were well-circumscribed areas in the superficial dermis with spindled and multinucleate giant cells arranged in a patternless pattern in a myxoid background. There were scattered dilated spaces occasionally lined by giant cells. There were increased vessels in some cases with hemorrhage and hemosiderin-laden macrophages. This process showed peri-adnexal extension. We performed immunohistochemical stains for CD34, CD21, CD1a, FXIIIa and CD117. The stromal cells in the GCF-like areas showed scattered positivity for CD117, increased positivity for FXIIIa and were negative for CD34, CD21 and CD1a. The giant cells were negative for all the stains. These findings suggest that this lesion adjacent to the encephalocele is not a GCF and may represent a mesenchymal reaction to the encephalocele.

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PARASAGITTAL CRANIAL FASCIITIS IN A TODDLER WITH INFRATEMPORAL FOSSA RHABDOMYOSARCOMA: ROLE OF RADIATION THERAPY. Eyas M. Hattab*, Joel C. Boaz, Thomas M. Ulbright and Annette C. Douglas-Akinwande. Indiana University Medical Center, Indianapolis, IN.

Cranial fasciitis (CF) is a rare lesion of young children characterized by proliferation of fibroblastic spindle cells. Most are scalp masses that erode the skull and occasionally involve the dura. They are only rarely reported intracranially and apart from one recent report (Longatti et al) have never been linked to radiation therapy (RT). We report a 32 month-old female toddler with a right infratemporal fossa rhabdomyosarcoma, diagnosed at 3 months of age, who was treated with surgery, chemotherapy and brachytherapy (4,140 cGy). A routine MRI of the brain at 23 months was normal. However, a subsequent imaging study 6 months later revealed a large, left parasagittal, dural-based mass with mass effect on the superior sagittal sinus and hyperostosis of the overlying bone. The lesion was hyperintense on T2 and FLAIR, hypointense on T1 and DWI sequences and it enhanced intensely. The radiographic features were consistent with a meningioma. The patient underwent open craniotomy and the mass was completely and readily excised. Histologically, the lesion was comprised of loosely and haphazardly arranged bland spindle cells embedded in a somewhat myxoid background. Thick hyalinized collagen bundles were especially prominent. The spindle cells reacted for vimentin but not SMA, myogenin, MyoD1 or EMA. A diagnosis of CF was rendered. This case is essentially identical to that reported by Longatti except that our patient received RT in the form of brachytherapy and not craniospinal irradiation. Brachytherapy effectively limits the radiation field raising questions about the role of RT in the pathogenesis of intracranial CF.

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ATYPICAL HIPPOCAMPAL HISTOLOGY DOES NOT CORRELATE WITH PRION PROTEIN POLYMORPHISM N171S IN TEMPORAL LOBE EPILEPSY PATIENTS. Linda W. Buckleair*[1], Dawna A. Armstrong[2], Daniel Yoshor[1], Eli Mizrahi[1] and G. Jackson. Snipes[1]. [1]Baylor College of Medicine; and [2]Texas Children's Hospital, Houston, TX.

Mesial temporal lobe epilepsy (MTLE) is frequently refractory to medications and temporal lobectomy may be required for seizure control. Ammon's horn sclerosis (AHS) is the most common lesion diagnosed in these patients, the majority of whom experience excellent control of their seizures. We have previously described atypical AHS histology, which is associated with poor seizure control following surgery (Epilepsia 2003;44(3):387-398). Walz et al. (Neurology 2003;61:1204-1210) recently demonstrated a variant allele of the prion protein gene (PRNP) in codon 171 (N171S) in 23% of their MTLE patients. Patients carrying this allele were five-times more likely to have seizure recurrence following lobectomy compared with those who did not. We hypothesized that our atypical AHS population might represent the same subset of patients carrying the N171S allele. We correlated histology with the presence or absence of this polymorphism hypothesizing that 20% of our MTLE cases would carry the N171S variant. **Materials and Methods:** Five cases of classic AHS, 5 cases of atypical AHS, and 5 tumor cases were selected for study. DNA was extracted from paraffin samples using the QIAamp mini-kit (Qiagen, USA), amplified by PCR, and purified using the QIAquick protocol (Qiagen, USA). Presence or absence of the N171S polymorphism was assessed by cycle sequencing (Seqwright, USA). **Results:** Sequencing confirmed successful isolation and amplification of the PRNP gene from all 15 samples. All 15 samples contained Asn (AAC) at codon 171. **Conclusions:** The atypical hippocampal sclerosis histology sometimes observed in our MTLE population does not correlate with the PRNP N171S polymorphism.

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ABNORMAL CEREBRAL HISTOGENESIS FOLLOWING DELETION OF DYSTROGLYCAN. Adam Ostendorf, Jakob Satz, Steven Westra, Susan E. Ross-Barta, Kevin P. Campbell and Steven A. Moore*. The University of Iowa, Iowa City, IA.

Some forms of congenital muscular dystrophy (CMD) exhibit cobblestone lissencephaly. Genetic and biochemical studies of these patients show abnormal glycosylation of dystroglycan (DG), while GFAP-Cre-loxP-mediated deletion of brain DG was shown previously to be sufficient to cause neuronal migration errors typical of CMD. The focus of this study is to evaluate DG-null mice during cerebral cortical histogenesis to better understand the pathogenesis of these brain malformations. By immunofluorescence, DG is reduced at E14.5 and absent by E15.5 in GFAP-$Cre^{+}/DG^{lox/lox}$ embryos. Meningeal heterotopia are first observed posteromedially at E14.5, and extend to anteriomedial cortex by E15.5 and 16.5. The lateral cortical surface remains normal in all GFAP-Cre embryos. By immunofluorescence, the glia limitans and radial glia are highly disrupted by E15.5 in regions with heterotopia. In nestin-$Cre^{+}/DG^{lox/lox}$ embryos, DG is absent by E13.5 when multifocal regions of migration abnormalities are first observed. Glia limitans/radial glia disruption and increasing size of heterotopia mimic GFAP-Cre mice yet occur earlier and eventually encompass a much larger portion of the cortex. In these tissue-selective models of DG deletion, DG is first absent from the glia limitans on embryonic days correlating closely with our earliest observations of migrational abnormalities. Earlier expression of Cre-recombinase leads to more extensive glia limitans/radial glia disruption and meningeal heterotopia. Some regions of cerebral cortex remain free of migration abnormalities despite the global deletion of DG suggesting that there is a critical stage of cortical development when DG is required to maintain glia limitans/radial glia integrity and/or support neuronal migration.

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WITHDRAWN

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EVIDENCE OF INCREASED CELL PROLIFERATION IN THE HIPPOCAMPUS IN EPILEPSY. Hidehiro Takei, Angus Wilfong, Daniel Yoshor, Dawna L. Armstrong and Meena B. Bhattacharjee*. Baylor College of Medicine, Houston, TX.

Background: Previous studies of Ammon's horn sclerosis (AHS) suggest that AHS is both the result of and the cause of seizures, and support the idea that seizures cause alterations in cell numbers, cell shape, and organization of neuronal circuitry. We hypothesized that epilepsy induces neurogenesis/gliogenesis. To test this hypothesis, we assessed hippocampal cell proliferation in AHS. **Design:** 12 resected hippocampal specimens in AHS patients (mean age 12.9 yrs, M/F: 5/7) and 11 autopsy controls (mean age 7.9 yrs, M/F: 6/5) were immunohistochemically stained for Ki-67 using the ABC technique. The total number of Ki-67 positive cells in each hippocampal area (dentate, CA-4, 3, 2, and 1 regions, subiculum) was independently counted by 2 pathologists. **Results:** Ki-67 positive cells, excluding endothelial cells, were observed in epilepsy and control cases. The cells were small and not obviously neuronal. Total numbers of Ki-67 positive cells in the hippocampi were significantly different between the AHS cases and controls, with the average of 21.5 and 5.3, respectively ($p = 0.04$). Significant differences were observed in the dentate gyrus ($p = 0.04$) and CA-4 region ($p = 0.02$) between the two groups. In the AHS cases, there were Ki-67 immunoreactive attached cells (cell clusters). Such cells were extremely rare in the control group. **Conclusions:** Compared with the controls, the AHS hippocampi have significantly higher cell proliferation, especially in the dentate and CA-4 region, with scattered cell clusters, suggesting the presence of active neurogenesis/gliogenesis and may be associated with some of the characteristic histological features seen in AHS (e.g. dentate dispersion).

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WEST NILE VIRUS ENCEPHALOMYELITIS IN THE SETTING OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) INFECTION. Kymberly A. Gyure. University of Maryland School of Medicine Baltimore, MD.

West Nile virus (WNV) produces a mild illness in the vast majority of infected patients. However, there is a growing body of evidence that the disease may be more severe in the immunocompromised population, and a number of cases in transplant recipients and in patients receiving chemotherapy have been described. We recently encountered a case of WNV encephalomyelitis in a HIV-positive individual. The patient, a 69-year-old man, presented to an outside hospital with confusion. Workup revealed positive WNV serology. His hospital course was complicated by aspiration pneumonia with respiratory failure requiring ventilator support, renal failure, and staphylococcal bacteremia. Two months after his initial presentation, he expired. A complete autopsy revealed an aortic dissection and acute bronchopneumonia with diffuse alveolar damage. The brain and spinal cord were grossly unremarkable apart from lacunar infarcts in the thalamus and pons. Microscopic examination demonstrated microglial nodules and perivascular chronic inflammation which were most prominent in the brain stem and spinal cord. The presence of WNV was confirmed by polymerase chain reaction using three separate WNV-specific primer/probe sets, and immunohistochemical staining for p24 antigen was negative. This case provides further support to the notion that WNV infection is a more virulent disease in immunocompromised patients and adds HIV infection to the list of potential underlying conditions.

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VARICELLA ZOSTER (VZV) ENCEPHALITIS: THE SPECTRUM OF CHANGES IN THREE BIOPSY SPECIMENS. Meenakshi Gupta[1], Mauricio Zapata[1], Stephen Hunter[1]*, Wun-Ju Shieh[2] and Sherif R. Zaki[2]. [1]Emory University School of Medicine and.[2]Centers for Disease Control, Atlanta, GA.

This 7-year-old girl suffered from an idiopathic chronic granulomatous disease treated with high dose corticosteroids. One week following onset of a typical zosteriform rash in a right C2 and C4 dermatome distribution, she developed ataxia. A solitary right pontomedullary lesion was biopsied, showing an acute necrotizing cerebritis with numerous neutrophiles and scattered macrophages and lymphocytes. Viral inclusions were absent, and numerous special stains for microorganisms were negative. One month later, MRI demonstrated extension of the brainstem lesion into the cerebellum and multiple, new predominantly subcortical, lesions in the cerebral hemispheres. A right frontal subcortical lesion was biopsied, showing a destructive white matter lesion with features of demyelination. Acute and chronic inflammation were sparse compared with the first specimen. Numerous eosinophilic intranuclear inclusions were present, predominantly at the periphery of the lesion. VZV immunostain was strongly positive both within the neuropil as well as within cell bodies and nuclei. Immunohistochemical testing was also positive on the first specimen. Immunostains for measles, herpes simplex and toxoplasmosis, as well as special stains for other microorganisms, were negative. The patient improved on acyclovir. One month later, a third biopsy was taken from deep white matter at the time of ventriculoperitoneal shunt placement. This specimen disclosed coagulative necrosis and prominent microvascular proliferation with minimal inflammatory infiltration, suggestive of recent infarction. Neither viral inclusions nor evidence of vasculitis were present. VZV immunostain showed focal rare positive staining foci. This study illustrates the spectrum of changes that may be encountered in VZV encephalitis.

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ROLE OF THE *APOE* GENOTYPE ON THE PHENOTYPIC EXPRESSION AND PARENCHYMAL AMYLOID-β PEPTIDE DEPOSITION IN GERSTMANN-STRÄUSSLER-SCHEINKER DISEASE ASSOCIATED WITH THE *PRNP* F198S MUTATION. Leticia Miravalle, Pedro Piccardo, Keiji Yamaguchi, Tony Perkins, Siu Hui, Ruben Vidal and Bernardino Ghetti. Indiana Alzheimer Disease Center, Indiana University, Indianapolis, IN. (Sponsored by Biagio Azzarelli*.)

Gerstmann-Sträussler-Scheinker disease (GSS) is a neurodegenerative disorder caused by mutations in the *PRNP* gene. GSS associated with the *PRNP* F198S mutation is pathologically characterized by the presence of prion protein (PrP) deposits and neurofibrillary tangles. In addition, amyloid-β (Aβ) has been found in elderly *PRNP* F198S mutation carriers. Individuals homozygous for valine at codon 129 (129VV) have an earlier age at onset than methionine-valine (129MV) heterozygous. The purpose of this study is to investigate the effect of the different *Apolipoprotein E* (*APOE*) genotypes on the neuropathologic phenotype of GSS-F198S-129VV and 129MV individuals and whether the *APOE*ε4 allele influences Aβ deposition in GSS-F198S. The *ApoE* genotype was determined in 15 deceased *PRNP* F198S mutation carriers. Statistical analyses were carried out using the Wilcoxon test. Neuropathological analyses were performed using antibodies against the amino-terminus of Aβ (Aβ3-6), Aβ peptides ending at residue 40 (AβX-40) and 42 (AβX-42). No statistically significant differences were observed when comparing the allelic distribution of *APOE* with the age at onset of clinical symptoms, disease duration and age at death in 129VV and 129MV individuals. Aβ deposition was seen in 7 of the 15 cases in association with older age and/or the presence of the *APOE*ε4 allele. Aβ deposits were immunopositive for Aβ3-6 and AβX-42. AβX-40 was not detected. Our data suggest that there is no significant effect of the *APOE* genotype on the three outcomes analyzed. The presence of the *APOE*ε4 allele and older age were seen associated with the deposition of AβX-42 peptides. Supported by P30AG10133.

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CHARACTERIZATION OF UBIQUITINATED INCLUSIONS IN FTD-MND-TYPE. Manjari Mishra[1,2], Tatjana Paunesku[1], Gayle E. Woloschak[1], Thomas Lukas[1], Teepu Siddique[1] and Eileen H. Bigio[1]*. [1]Northwestern University Feinberg School of Medicine, Chicago, IL. [2]Northwestern Alzheimer Disease Center, Chicago, IL.

There is mounting evidence that neurodegenerative disorders share a process of protein accumulation and deposition, such as tau in tangles and β-amyloid in plaques in AD and alpha-synuclein in Lewy bodies in Parkinson disease and DLB. FTD of the motor neuron disease type (FTD-MND type) contains ubiquitinated inclusions in hippocampal dentate neurons and neuroglial cells in cortical layers II and III. However, it remains virtually the only dementia with inclusions that are uncharacterized with respect to their protein composition. With the goal of identifying this protein, we analyzed the genomic profile by Affymetrix HGU133 Plus 2.0 microarrays and the proteomic profile by HPLC-coupled mass spectrometry and MALDI-TOF in tissue homogenates from superficial frontal cortex samples of 2 cases of FTD-MND-type, 2 cases of FTD-MND, and 3 age-matched controls. Western blots of FTD-MND-type probed with ubiquitin showed a 180kD protein. Microarrays analysis showed differential expression of genes in FTD-MND-type and controls in over 100 gene networks, including genes involved in cell-to-cell signaling, ubiquitin-proteasome, and apoptosis networks. Mass spectrometry showed changes in synapse-related, cytoskeletal protein/filament-related, microtubule-axon-related, and ubuiquitin-proteasome-related proteins. We plan to confirm the differentially expressed genes using real-time PCR and Northern blots. Our future goals are to address the causes and effects of the protein aggregation, significance of the location and altered protein expression patterns of the inclusions, their association with neuronal dysfunction in this disorder if it exists, and the potential relationship of these inclusions to neurogenesis.

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ADULT ONSET LEUKODYSTROPHY WITH NEUROAXONAL SPHEROIDS (AOLNAS), REPORT OF THREE CASES. S. H. Freeman, D. Tian, M. P. Frosch and E. T. Hedley-Whyte*. Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Adult onset leukodystrophy is a rare entity with both hereditary and sporadic forms. Two overlapping forms with variable clinical features involving both motor and cognitive changes have been described. Pigmented orthochromatic leukodystrophy (POLD) described by Van Bogaert and Nyssen (1936) characterized by pigmented macrophages, and hereditary diffuse leukoencephalopathy (HDLS) described by Axelsson (1984). The neuropathologic changes described in AOLNAS include degeneration of white matter with loss of myelin sheaths and axons, gliosis, macrophages, and neuroaxonal spheroids (Marotti 2004). We report three cases, two autopsies and one biopsy, of AOLNAS encountered at the MGH in the past five years. All three subjects were females ranging in age from 34-50 years. The two autopsied patients had a 4-5 year history of cognitive decline. The brain biopsy was from a 50 year-old with approximately 1 year of behavioral changes and cognitive decline. All three patients demonstrated white matter abnormalities on MRI. All three brains exhibited severe leukodystrophy with both myelin and axonal loss, and large numbers of axonal spheroids. Macrophages with and without pigment were also present. The U-fibers, cerebral cortex, and cerebellum were all spared. The only difference in the biopsy was that the damaged white matter had ill-defined borders. The cerebral cortex was unremarkable, and the axonal spheroids were strongly neurofilament positive. We conclude that the diagnosis of AOLNAS can be made by biopsy, and that the ill-defined borders of the white matter involvement reflect an earlier stage of the disease. (Supported by AG005134).

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PROLONGED FORMALIN FIXATION IMPAIRS IMMUNOHISTOCHEMICAL STAINING FOR ALPHA-SYNUCLEIN IN BRAINS OF PATIENTS WITH LEWY BODY DISEASE. Tiffany M. Bauer[1], Christa L. Hladik[2] and Charles L. White, III*[2]. [1]Binghamton University, Binghamton, NY. [2]University of Texas Southwestern Medical School, Dallas, TX.

Formalin fixation is widely used for preservation of human tissues for diagnostic and research studies. Longitudinal studies examining the effects of long-term formalin fixation on immunohistochemical staining for abnormal proteins found in diseased tissues are scarce. We undertook this study to investigate the possible effects of prolonged formalin fixation on immunoreactivity of alpha-synuclein, a pre-synaptic protein abnormally expressed in Lewy body disease (LBD), a group of neurodegenerative diseases that includes idiopathic Parkinson disease and Lewy body variant of Alzheimer disease (LBVAD). LBD is characterized pathologically by the presence of alpha-synuclein-immunoreactive Lewy bodies and Lewy neurites (LN) in brain tissue sections. To determine if a relationship exists between length of exposure to formalin and LN density, we examined the anterior cingulate gyrus (ACG) in autopsy brains from 24 patients with pathologically verified LBD. LN density in ACG tissue stored in formalin for 1-12 years was compared with LN density in ACG archived in paraffin blocks since the time of autopsy. Tissues were immunostained with monoclonal antibody LB509 to human alpha-synuclein and differences in LN density between the formalin and paraffin archival tissues were quantified using computer-assisted image analysis. We found that LN density declined dramatically after 4 years of storage in formalin. These findings suggest that accurate interpretation of quantitative immunohistochemical data from studies of autopsy tissues must take tissue storage conditions into account.

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FINE STRUCTURE OF INTRANUCLEAR INCLUSIONS IN NEURONAL INTERMEDIATE FILAMENT INCLUSION DISEASE. Sabrina Mosaheb[1], Lily Hashemzadeh-Bonehi[1], Stuart L. Rulten[1], John E. Kay[2], Julian R. Thorpe[1] and Nigel J. Cairns[3]*. [1]School of Life Sciences, University of Sussex, Brighton, UK. [2]Brighton & Sussex Medical School, Brighton, UK. [3]Department of Neurology, Washington University School of Medicine, St. Louis, MO.

Introduction: Neuronal intermediate filament (IF) inclusion disease (NIFID) is a neurological disease with a clinically heterogeneous phenotype, including frontotemporal dementia, pyramidal and extrapyramidal signs presenting at a young age. The pathological signature lesions of this disease are neuronal IF cytoplasmic inclusions which contain type IV IF proteins, which contain neither tau nor alpha-synuclein, but are variably ubiquitinated. In some cases, neuronal intranuclear inclusions (NIIs), which are ubiquitinated, but do not contain IF proteins, have also been observed at the light microscope level. **Materials and Methods:** Tissue from the frontal lobe of four cases of NIFID with both NII and CI was selected. Transmission immunoelectron microscopy (TEM) was used to investigate the fine structure of inclusions using a panel of antibodies to IF proteins, ubiquitin, and polyglutamine. **Results:** Neuronal intranuclear inclusions contained a mixture of filamentous and granular material and NII filaments were broader (19.87 $\pm$ 0.72 nm) than those of the CIs (9.87 $\pm$ 0.35 nm). Using immunogold labelling TEM, we confirmed the presence of ubiquitin, neurofilament and α-internexin proteins in CIs; while NIIs contained ubiquitin epitopes, but not IF proteins. Additionally, poly-CAG-containing proteins were absent from NIIs. **Conclusions:** This is the first demonstration of the fine structure of NIIs in NIFID. These data indicate that ubiquitin epitopes are present within the pathological protein aggregates in the nucleus and that ubiquitination may precede, or be independent of, the accumulation of neuronal IFs in CIs in NIFID.

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DISTINCTIVE 4R TAUOPATHIES IN TWO SIBLINGS. Keith A. Josephs, Joseph E. Parisi*, Bradley F. Boeve and Dennis W. Dickson*. Mayo Clinic, Rochester, MN and Jacksonville, FL.

Familial neurological disease with diverse and distinctive pathologic features may be more common than previously reported. A 71-year-old man (proband) and his 68-year-old sister suffered from a progressive neurodegenerative dementia with features suggesting frontotemporal lobar degeneration. Autopsy examination demonstrated distinct 4R tauopathies in both. In the proband, tau-positive argyrophilic grains involved the medial temporal lobe, but also extended throughout the temporal lobe, and to a lesser extent frontal and parietal lobes, basal ganglia and brainstem. There were many neocortical "ballooned" neurons, as well as mild neuronal loss, spongiosis and gliosis, and numerous "coiled bodies" in the medial temporal lobe. In the sister, there were widespread "ballooned" neurons and extensive glial pathology with numerous astrocytic plaques. The pathological diagnosis in the proband was diffuse argyrophilic grain disease (DAGD), while that in the sister was corticobasal degeneration (CBD): Diffuse AGD is a recently described variant of 4R tauopathy and different from limbic AGD. Both DAGD and CBD are rare pathologies; their occurrence in these siblings may suggest a common etiopathogenesis with genetic underpinnings.

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MULTIPLE SYSTEM ATROPHY WITH PICK-LIKE BODIES PRESENTING AS FRONTOTEMPORAL DEGENERATION: TWO CASES ILLUSTRATING THE CRITICAL DIAGNOSTIC ROLE OF ALPHA-SYNUCLEIN IMMUNOHISTOCHEMISTRY. Cheryl Lund[1], James M. Powers[1], Philip J. Boyer[2]*, Myron F. Weiner[2], Anne M. Lipton[2], Charles L. White[2] and Dennis W. Dickson[3]. [1]University of Rochester, Rochester, NY. [2]University of Texas Southwestern Medical Center, Dallas, TX. [3]Mayo Clinic, Jacksonville, FL.

Background: Multiple system atrophy (MSA) is a sporadic neurodegenerative disorder characterized by parkinsonism, cerebellar signs and autonomic dysfunction, but preserved cognition. At autopsy, striatonigral and olivopontocerebellar degeneration is common, but cerebral and limbic system pathology is minimal. **Objective:** To report the clinical and pathologic features of two cases of MSA with circumscribed temporal atrophy and histopathologic features suggestive of Pick's disease. **Methods:** Clinical features, neuropathology and immunohistochemistry of two cases are reviewed. **Results:** Case 1 was a 73-year-old woman with a 7-year history of slowly progressive dementia, with depression and personality change, and later developing parkinsonism consistent with frontotemporal dementia. Case 2 was a 70-year-old woman with a 3-year history of progressive motor and cognitive dysfunction clinically consistent with corticobasal degeneration with asymmetrical tremor, myoclonus, apraxia, dysarthria and parkinsonism. She also had hyperreflexia, ataxia and terminal dementia. Neither case had autonomic dysfunction. Both cases had medial temporal lobe atrophy, with small and discolored hippocampus and amygdala. Case 2 had atrophy and brown discoloration of the putamen. Both had depigmentation of the substantia nigra. Gallyas stains revealed Pick body like inclusions in the hippocampus, including dentate fascia, that were negative (case 2) or variably positive (case 1) for tau, but intensely positive for alpha-synuclein. Glial cytoplasmic inclusions that were Gallyas- and synuclein-positive were detected in basal ganglia, brainstem and cerebellum. **Conclusions:** These cases illustrate that MSA may present with frontotemporal lobar degeneration, rather than striatonigral or olivopontocerebellar degeneration. Synuclein immunohistochemistry is important in the differential diagnosis of frontotemporal degenerations.

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MULTIPLE SYSTEM TAUOPATHY WITH PRESENILE DEMENTIA (MSTD): DOES THE MAPT HAPLOTYPE INFLUENCE THE CLINICAL PHENOTYPE? Salvatore Spina, Martin R. Farlow, Frederick W. Unverzagt, Bernardino Ghetti* and Jill R. Murrell. Indiana Alzheimer Disease Center, Indiana University, Indianapolis, IN.

The deposition of hyperphosphorylated tau protein in neurons and glia constitutes the pathological hallmark of MSTD, a disease associated with frontotemporal dementia, parkinsonism and the E10+3 intronic MAPT mutation. A series of polymorphisms have been described in MAPT that have been used to define two extended haplotypes (H1 and H2), which are in complete linkage disequilibrium. Twenty-nine individuals from the MSTD family were genotyped to determine if the MAPT haplotype influenced phenotypic heterogeneity in MSTD. Genomic DNA was extracted from frozen or paraffin-embedded brain tissue or peripheral blood. The haplotype was determined by amplification of a 238 bp deletion in intron 9 of MAPT. This deletion is inherited as part of the H2 haplotype. Twenty individuals had an H1H2 haplotype and nine presented an H2H2 haplotype. Among those with H1H2, 15 have presented symptoms of MSTD: their age at onset is 48.1 $\pm$ 5.4 years and the disease duration (n = 9) is 10.1 $\pm$ 5.7 years. Among those with H2H2, 7 have presented symptoms of MSTD: their age at onset is 48.1 $\pm$ 5.8 years and the disease duration is (n = 5) 10.6 $\pm$ 2.4 years. Information on the presenting clinical signs is available for 14 H1H2 and 7 H2H2 patients: personality changes (78% vs 57%), memory loss (35% vs 57%), dysexecutive symptoms (42% vs 14%) and dizziness (7% vs 14%). While the MAPT haplotype does not appear to affect the age at onset and disease duration, it may influence clinical presentation of the disease. P30 AG10133.

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PARKINSONIAN TAUOPATHY WITH UNILATERAL NEUROFIBRILLARY DEGENERATION OF THE MEDIAL TEMPORAL LOBE: AMYLOID-INDEPENDENT NEUROFIBRILLARY PATHOLOGY. Rudy J. Castellani* and Chris Vincent. Michigan State University, East Lansing, MI.

Objective: A patient with widespread tau pathology that included unilateral Alzheimer-like neurofibrillary degeneration of the right medial temporal lobe is presented. **Methods:** A 58 year-old man presented with parkinsonism, tangential speech, impaired judgment, and bilateral upward gaze palsy. Worsening tremor, increased rigidity, torticollis, and cognitive decline ensued. The patient expired 3 years after presentation. The brain was examined at autopsy. **Results:** The most marked loss of neurons affected the pars compacta of the substantia nigra, the subthalamic nucleus, and the right hippocampus/subiculum. Residual neurons in the substantia nigra and subthalamic nucleus often contained globose neurofibrillary tangles. Inclusions of varying morphology were also present in the oculomotor nucleus, colliculi (rare), periaqueductal gray, and mesencephalic and pontine raphe. No significant pathology was observed in the medulla or cerebellum, including the dentate nucleus. The cerebral cortex contained only isolated ballooned neurons in the pericentral region. The right hippocampal formation, subiculum, and entorhinal cortex demonstrated numerous neurofibrillary tangles, with extracellular "ghost" tangles. No neurofibrillary tangles were observed in the left medial temporal lobe. Phospho-tau immunohistochemistry demonstrated widespread neuronal and glial tau pathology. Extenstive phospho-tau reactivity was present in the right medial temporal lobe while no significant immunostaining was present on the left. Amyloid beta-positive diffuse plaques were evenly distributed in the cerebral cortex bilaterally. **Conclusion:** While many conditions are associated with neurofibrillary tangle formation, unilateral neurofibrillary degeneration that resembles advanced Alzheimer's disease is unusual, and suggests the possibility that neurofibrillary degeneration occurs independent of amyloid-beta deposits in some cases.

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NEUROPATHOLOGIC HETEROGENEITY OF PICK'S DISEASE. Masaki Takao[1,2,*], Jill R. Murrell[1], Keiji Yamaguchi[1], Frederick W. Unverzagt[1], Bradley S. Glazier[1], Martin R. Farlow[1], Jordan Grafman[3] and Bernardino Ghetti[1,*]. [1]Indiana Alzheimer Disease Center, Department of Pathology and Laboratory Medicine, Department of Psychiatry, Department of Neurology, Indiana University School of Medicine, Indianapolis, IN. [2]Mihara Memorial Hospital, Isesaki, Gunma, Japan. [3]Cognitive Neuroscience Section, National Institute of Neurological Disease and Stroke, Bethesda, MD.

The characteristic pathologic changes in Pick disease (PiD) are well known. We present atypical findings from two individuals diagnosed neuropathologically as having PiD. **Case 1:** A 64-year-old female developed progressive deterioration of cognitive function. At age 66, data from clinical and neuropsychological evaluation were consistent with primary progressive aphasia. MRI and CT showed cerebral atrophy predominantly of the left frontal and temporal lobes. She died at age 73. The brain weighed 993 grams and atrophy was prominent in the left frontal and temporal gyri as well as the head of the caudate nucleus and corpus callosum. A prominent histologic finding was the wide distribution of Pick bodies (PBs) that were numerous in the frontal, temporal, parietal and occipital cortices as well as the hippocampus and basis pontis. Numerous tufted astrocytes (TAs) were also present in the cerebral cortex. PBs and TAs were immunoreactive using AT8 antibody, but immunonegative using 12E8. **Case 2:** A 65-year-old female developed behavioral changes. Data from clinical and neuropsychological examination were consistent with PiD. Parkinsonism was not recorded. She died at age 70. The brain weighed 1100 grams and atrophy was prominent in the frontal and temporal lobes, bilaterally. Numerous PBs were present in the frontal and temporal cortices and hippocampus. In addition, α-synuclein immunopositive Lewy bodies and neurites were observed in the substantia nigra, locus coeruleus and substantia innominata. In order to determine whether these pathologic findings are atypical of PiD, a retrospective analysis using antibodies against tau and α-synuclein would be needed. P30 AG10133.

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IDENTIFICATION OF AGGREGATE-INTERACTING PROTEINS AND PROTEASOME INVOLVEMENT IN TRANSGENIC MICE EXPRESSING A C-TERMINAL MUTANT OF THE FERRITIN LIGHT POLYPEPTIDE GENE. Ruben Vidal*, Yingbin Su, Xiaoying Gao, Rose M. Richardson, Leticia Miravalle and Bernardino Ghetti. Indiana Alzheimer Disease Center, Indiana University, Indianapolis, IN.

Mutations in the coding sequence of the ferritin light chain (FTL) gene result in a neurodegenerative disease characterized by the intranuclear and intracytoplasmic accumulation of ferritin and iron in cells of the central nervous system. The aim of this study is to investigate the molecular mechanisms by which mutant ferritin may cause disease using a transgenic (Tg) mouse model that over-express the FTL498-499InsTC mutation. We investigated i) whether ferritin nuclear inclusions (NIs) sequester nuclear proteins such as transcription factors, leading to aberrant transcriptional regulation and ii) whether abnormal accumulation of ferritin is associated with incomplete ubiquitin-dependent proteolysis, that may result in cell toxicity. Control and FTL-Tg mice were studied neuropathologically and biochemically. We determined that CREB-binding protein (CBP), a transcriptional coactivator, is sequestered from its normal nuclear localization early in the formation of NIs and is found with ferritin in the SDS-insoluble protein fraction isolated from the brain of FTL-Tg mice. Immunohistochemical and biochemical analyses showed that ferritin deposits are ubiquitin-positive and contain several elements of the 20S proteasome such as the 11S proteasome regulator protein (PA28α) and the 19S regulator ATPase subunit 6b (Tbp7). The 19S subunit is strongly associated with ferritin inclusions since it can be immunoprecipitated from brain homogenates using antibodies against ferritin. No evidence of SUMOylation was observed. Our results strongly suggest that at least two key toxic mechanisms that involve the gain of a toxic function by ferritin may be implicated in the disease. Supported by Alzheimer's Association, P30AG10133.

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FAMILIAL FRONTO-TEMPORAL LOBAR DEGENERATION WITH UBIQUITIN POSITIVE, TAU NEGATIVE INCLUSIONS (FTD-U), WITH MARKED NEURONAL LOSS IN THE STRIATUM, THALAMUS AND SUBSTANTIA NIGRA, AND EXTENSIVE HIPPOCAMPAL SCLEROSIS. Ada Baisre, Manuel A. Cruz, Leroy R. Sharer and Eun-Sook Cho*. UMDNJ-New Jersey Medical School, Newark, NJ.

We are reporting pathological findings in one of three sisters, all of who had a clinical course typical of fronto-temporal dementia. Each woman developed symptoms in her early sixties and died 7 to 8 years later. The two older sisters had no post-mortem examination. At the age of 63 the current patient showed cognitive decline with emotional withdrawal and decreased communication. Within four years she became mute except for echolalia. She died at a nursing home at the age of 70. The brain weight was 790 gm., with extensive symmetric atrophy of the fronto-temporal lobes, dilated ventricles, and pale substantia nigra. Microscopically the involved cortex showed a moderate degree of neuronal loss and microvacuolation of the neuropil in the upper layers. Extensive neuronal loss was noted in the caudate nucleus, in the medial portion of the putamen, in the anterior nucleus of the thalamus, and in the medial two thirds of the substantia nigra. Marked hippocampal sclerosis was present involving CA1 and the subiculum. Immunohistochemical studies revealed ubiquitin positive, tau and synuclein negative inclusions in the cytoplasm of scattered small neurons in the upper layers of the frontal cortex, and intranuclear inclusions in many granular cells in the dentate gyri of the hippocampus. At least one similar kindred has been reported with an autosomal dominant mode of inheritance.

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CALRETININ IMMUNOHISTOCHEMISTRY IN NEURODEGENERATIVE DISORDERS ASSESSED USING TISSUE MICROARRAYS. Linda W. Buckleair*[1] and Suzanne Z. Powell[2]. [1]Baylor College of Medicine and [2]The Methodist Hospital, Houston, TX.

Background: The relatively new technique of tissue microarrays (TMA's) is making the process of evaluating large numbers of cases for immunohistochemical expression more efficient and reproducible. Unlike tumor research, this valuable technique has not been widely applied to neurodegenerative disease (NDD). We constructed TMA's of NDD for the purpose of high throughput immunohistochemical screening for expression of antigens such as calretinin that have been incompletely explored by conventional means. Our initial results are promising, and confirm the utility of the technique in the evaluation of neurodegenerative disease. **Materials and Methods:** A total of 250 blocks from 54 neurodegenerative disease autopsy cases: Alzheimer disease (n = 30), Lewy body dementia (n = 5), dementia lacking distinctive histology (n = 3), amyotrophic lateral sclerosis (n = 3), progressive supranuclear palsy (n = 1), diffuse tauopathy (n = 3), neurological disease controls (n = 4) and age-matched controls (n = 5) were selected from archival material. Cores (0.6 mm) were manually taken from standardized sites (cortex, hippocampus, basal ganglia, substantia nigra) and sites of maximal pathology. Cores from the donor blocks were assembled into a recipient block with control tissue cores (lung). Upon completion, the recipient block was cut and stained for calretinin immunohistochemical staining. **Results:** Evaluation of calretinin immunohistochemical staining shows mainly diffuse neuropil and scattered cortical neuron staining, without staining in the pigmented neurons of the midbrain. **Conclusion:** This study confirms previously described expression patterns of this protein in these diseases, but was performed rapidly in a large number of cases using a single slide.

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PRESENCE OF LEWY BODIES IN PROGRESSIVE SUPRANUCLEAR PALSY REPRESENTS AN INDEPENDENT DISEASE PROCESS. Hirotake Uchikado and Dennis W. Dickson*. Mayo Clinic, Jacksonville, FL.

Background: Progressive supranuclear palsy (PSP) is a neurodegenerative tauopathy characterized by parkinsonism, vertical supranuclear ophthalmoplegia and early falls. In previous studies Lewy bodies (LBs) were detected in about 10 percent of PSP cases. **Objectives:** To investigate the frequency of LBs in PSP and to study the density and distribution of LBs, including LB stage, in PSP/LBD compared to pure LB disease (LBD). **Methods:** 290 cases of PSP were screened for synuclein pathology with immunohistochemistry; 31 cases (10.7%) had LBs. Two cases were excluded from further study, because of concurrent Alzheimer's disease. 24 cases of LBD were compared to the 29 cases of PSP/LBD. **Results:** The age, sex, Braak stage and density of senile plaques did not differ between LBD and PSP/LBD. The distribution of LBs according to the LB staging scheme of Braak and coworkers was also similar. The density of LBs in subcortical nuclei, but not in the amygdala, was greater in LBD than in PSP/LBD. The disease duration was greater in LBD than PSP/LBD. Substantia nigra neuronal loss was more severe in PSP/LBD than LBD, but not different in other nuclei studied. **Conclusions:** The findings suggest that LBs in PSP represent an independent disease process, with distribution of LBs similar to pure LBD. The greater density of LBs in LBD compared with PSP/LBD could be due to the longer disease duration in LBD, and the greater neuronal loss in the substantia nigra in PSP/LBD may be related to vulnerability of this brain region to both disease processes.

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ULTRASTRUCTURAL STUDIES OF HEREDITARY DIFFUSE LEUKOENCEPHALOPATHY WITH SPHEROIDS. Wen-Lang Lin[1], Yasuhiko Baba[1], Zbigniew K. Wszolek[1], Michael S. Handler[2] and Dennis W. Dickson[1]*. Mayo Clinic, Jacksonville, FL. [2]Neuropathology & Forensic Pathology Consulting, Inc., Overland Park, KS.

Background: Hereditary diffuse leukoencephalopathy with spheroids (HDLS) is an autosomal dominant disorder characterized by progressive cerebral white matter degeneration with axonal spheroids. A limited number of ultrastructural studies have been reported of this rare disorder. **Objectives:** Immunohistochemical and ultrastructural features are described in two unrelated, 46 and 55 year-old women. **Methods:** Periventricular white matter from the frontal lobe was obtained from formalin-fixed tissue and was processed for embedding in LR White resin for immunoelectron microscopy or embedding in Epon 812 for routine EM. **Results:** Light microscopic studies showed cerebral myelin and axonal loss with axonal spheroids that were immunopositive for amyloid precursor protein (22C11) or phosphorylated neurofilament (pNF; SMI-31). A subset was positive for ubiquitin. The overlying cortices had ballooned neurons that were positive for pNF and αB-crystallin (αBc). The spheroids were negative or weakly stained for ubiquitin and αBc. Ultrastructurally there were two types of spheroids – some filled with dense aggregates of pNF and others filled with membranous dense bodies and vesicles with fewer filaments. Nonphosphorylated NFs (SMI-32) were also present in some spheroids. They were negative for αBc. Spheroids had very thin and discontinuous myelin sheaths. Bizarre astrocytes that were double labeled for αBc and glial fibrillary acidic protein, and lipid- and myelin-laden macrophages were present. **Conclusions:** The presence of two distinct types of axonal spheroids has not been emphasized in previous reports of HDLS. It remains to be determined if the spheroids are a reactive process or a primary mechanism of white matter degeneration.

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COEXISTENCE OF PSP AND MSA. Hirotake Uchikado and Dennis W. Dickson. Mayo Clinic, Jacksonville, FL. (Sponsored by Steven G. Younkin*.)

Background: Progressive supranuclear palsy (PSP) is characterized by parkinsonism, vertical supranuclear ophthalmoplegia (VSO) and early falls. The neuropathology is characterized by neurofibrillary tangles, tufted astrocytes and coiled bodies, but some brains show other neurodegenerative lesions, including senile plaques and Lewy bodies. The presence of alpha-synuclein-immunoreactive glial cytoplasmic inclusions (GCI) typical of multiple system atrophy (MSA) has not been addressed in PSP. **Objective:** To investigate of the frequency of GCI in PSP and to report the clinical and pathological features of a case of PSP with concomitant MSA. **Methods:** 290 cases of PSP were screened for synuclein pathology using an antibody to alpha-synuclein and immunohistochemistry. **Results:** One case (0.3%) had neuropathologic findings consistent with both PSP and MSA. This patient was an 86-year-old woman who began falling unexpectedly at age 74, which was followed by loss of dexterity of fingers, dysphagia and dysarthria, as well as memory impairment. Neurologic examination revealed mild blepharospasm, VSO and bradykinesia consistent with PSP. She had no documented dysautonomia or cerebellar signs. Pathological examination showed neuronal and glial lesions consistent with PSP, as well as plaques and tangles consistent with concurrent Alzheimer's disease (AD). Synuclein immunostains showed GCI and neuronal inclusions in a distribution typical of MSA. There was also evidence of cerebrovascular pathology. Double-immunolabeling studies showed no co-localization of synuclein and tau in neuronal and glial lesions. **Conclusions:** Based upon the findings in this case, the neuropathologic changes of PSP and MSA, as well as AD, are distinct and independent, but they can occasionally coexist.

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PROTEIN AGGREGATION IN MUSCLE FIBERS AND THEIR DISEASES. Hans H. Goebel*. Department of Neuropathology, Johannes Gutenberg University–Medical Center, Mainz, Germany.

Background: Protein aggregation is defined by focal or multifocal rather than diffuse accumulation of proteins in the sarcoplasm of myofibers. **Objectives:** Assessing the nosological, i.e. clinical, morphological, biochemical, and molecular spectrum of protein aggregation in muscle fibers and their diseases, protein aggregate myopathies (PAM). **Material and Methods:** Based on biopsied muscle tissue, inclusions in PAM have been analyzed by immunohistochemistry and immunoelectron microscopy. **Results:** Inclusions such as cytoplasmic or spheroid bodies, tubulofilamentous material, hyaline bodies, nemaline or rod bodies, and aggregates of actin filaments have been linked to certain CM. Currently, PAM encompass desminopathies, myotilinopathies, selenoproteinopathies, hereditary (h-IBM) and sporadic (inflammatory) inclusion body myopathies, actinopathy, and myosinopathy. **Conclusion:** Among the different PAM certain forms seem to be hereditary, marked by mutant proteins and others appear as acquired forms. Two types of PAM may be distinguished, one affecting catabolic metabolism of extralysosomal proteins in DRM and h-IBM while actinopathies and myosinopathy suggest impairment of maturational integration of proteins in normal sarcomeres.

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NERVE DEGENERATION AND ITS ASSOCIATION WITH PERINEURAL INVASION AND INFLAMMATION IN SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK REGION. Ghoushia Rizvi, Jahanara Zahid and Cunfeng (Frank) Pu*. Department of Pathology, Hershey Medical Center, Pennsylvania State College of Medicine, Hershey, PA.

Squamous cell carcinoma (SCC) is a common malignant tumor in the head and neck region. Clinically, patients with this tumor sometimes show evidence of cranial polyneuropathy, manifested as paralysis, numbness, and pain. The focus of this research is to demonstrate the pathological changes in the nerves and their association with perineural invasion (PNI) and inflammation in SCC of the head and neck region. In the retrospective study, 11 cases of SCC with pathological evidence of PNI in the head and neck region were used. Immunohistochemistry with S100, neurofilament, CD45RB, CD3, and CD20 were employed. The results were studied under the light microscope. The relative density of the axons was graded using a 3-point scale: normal (-), mild loss (+), relative density of the axons (++), and marked loss (+++). The results showed there were moderate to marked loss of axons in the nerves with PNI. There were also varied degrees of lymphocytic infiltrate, including both T and B type lymphocytes, in the tumor and its surrounding soft tissue; however, only rare lymphocytes were present in the nerves, and there was no difference in the number of lymphocytes in the nerves with or without axonal degeneration. The results indicate that PNI in the SCC of the head and neck region causes axonal degeneration, and the axonal degeneration is not caused by inflammation.

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PAINLESS LUMBOSACRAL PLEXOPATHY: A CASE OF NEUROMUSCULAR CHORISTOMA. Brian H. Le, Sharon Rivas, Zachary Simmons, Kimberly Harbaugh, Javad Towfighi and Cunfeng (Frank). Pu*. Pennsylvania State University College of Medicine, Hershey, PA.

Lumbosacral plexopathies can be attributed to multiple etiologies including neoplasms, radiation effects, retroperitoneal hemorrhage or abscess, diabetic neuropathy, trauma, injections, or may be idiopathic. This case study demonstrates an unusual etiology of lumbosacral plexopathy in a 28-year-old male who presented for evaluation of painless left leg and foot atrophy, which was brought to attention following minor sports-related injury to the limb. Nerve conduction studies revealed an absence of medial and lateral plantar and sural sensory responses, with decreased peroneal and tibial motor responses. EMG demonstrated acute denervation changes. MRI studies revealed diffuse thickening of the left lumbosacral plexus and sciatic nerve. At surgery, the left sciatic nerve was enlarged to at least three times normal size, demonstrating firm texture with pink-red coloration. Paraffin embedded sections revealed mature skeletal muscle fascicles admixed with disorganized nerve fascicles. In some areas, muscle fascicles were surrounded by loosely arranged nerve fibers; in other areas, individual muscle fibers were embedded within endoneurial connective tissue. The skeletal muscle component stained for antibodies to desmin, and the neural components demonstrated positive staining for S-100 and collagen IV. Based on these features, the diagnosis of neuromuscular choristoma (hamartoma) was rendered. Neuromuscular choristoma is a rare entity for which the natural history is not fully understood. To date there are 19 reported cases, six of which involved the sciatic nerve; however, unlike the current case, none of them involved the lumbosacral plexus.

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SKELETAL MUSCLE INVOLVEMENT IN NEPHROGENIC FIBROSING DERMOPATHY. Philip J. Boyer[1]*, Cherise Cortese[2], Matthew Kershisnik[3], Brian H. Le[4], Mark C. Tekrony[5], Charles Joseph[6] and Shawn Cowper[7]. [1]University of Texas Southwestern, Dallas, TX. [2]St. Louis University, St. Louis, MO. [3]Logan Regional Medical Center, Logan, UT. [4]Penn State University, Hershey, PA. [5]University of Virginia, Charlottesville, VA. [6]Neurology Associates of Lynchburg, Lynchburg, VA. [7]Yale University, New Haven, CT.

Background: Nephrogenic fibrosing dermopathy (NFD) is an emerging dermatologic entity of unknown pathogenesis, seen in the setting of chronic renal failure, with or without a history of dialysis. **Objective:** Characterize muscle involvement in NFD. **Methods:** Muscle involvement in NFD was assessed in specimens obtained from (a) skin biopsy which included superficial muscle (N = 1) and (b) superficial and deep sites obtained at autopsy (N = 3). Sections from all specimens were examined histologically and, when possible, histochemically using a broad stain and histochemical panel and by immunohistochemistry for CD34, S100, and SMI 31. **Results:** In all regions of muscle underlying involved skin, bands of dense connective tissue populated by plump CD34- positive cells extended from the dermis and subcutis into the epimysium, perimysium, and endomysium. In contrast, specimens from deep muscle contained rare plump CD34 positive cells but lacked a fibrotic response except in areas perturbed by previous surgical intervention where the fibrosis was identical to that near the skin. In all involved areas, blood vessels and nerve branches and twigs were enveloped by dense fibrosis. There was variable evidence of active and chronic neurogenic change, most severe in association with regions of fibrosis but also notable in deep specimens. An element of type 2 atrophy was noted in some specimens. Myopathic changes were not prominent. **Conclusions:** Findings suggest an incidental rather than primary effect of NFD on skeletal muscle. Neurogenic findings could be the result of nerve twig impingement by fibrosis or neuropathy secondary to renal failure or other comorbid processes.

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EXTENSIVE MACROPHAGIC MYOSITIS IN DELTOID AND TRABECULAR MYOPATHY IN QUADRICEPS IN A PATIENT WITHOUT ANTECEDENT VACCINATION. Eun-Sook Cho[1]*, Ada Baisre[1], Manuel A. Cruz[1], Steven Rosner[2] and Amyn Rojiani[3]. [1]UMDNJ-New Jersey Medical School, Newark, NJ. [2]Pascack Valley Hospital, Westwood, NJ. [3]University of South Florida College of Medicine, Tampa, FL.

A 65 year-old man developed polyarthritis, intermittent fever, and pericardial and pleural effusions. Rheumatoid factor was elevated, and a work-up for an infectious etiology was negative. The patient was treated with prednisone with limited improvement. Progressive proximal muscle weakness was reported, and wasting was noted on examination. There was no elevation of serum creatine kinase. The patient denied any recent vaccination. Two random muscle biopsies were obtained, one from the right quadriceps and the other from the right deltoid. In the deltoid biopsy an extensive infiltrate of non-epithelioid macrophages was present in the fascia, epimysium and perimysium. No other inflammatory cells were seen except for scattered CD3+ lymphocytes around blood vessels. Special stains did not reveal any infectious agents, and energy dispersive X-ray analysis did not identify any abnormal aluminum deposits. There were scattered necrotic and regenerating muscle fibers was seen. A trabecular pattern was present with NADH-Tr reaction in approximately 20% of fibers. The quadriceps biopsy had trabecular pattern in about 60 % of muscle fibers, but no macrophagic infiltrates. In addition mild type 1 fiber predominance and type 2 fiber atrophy were noted. Macrophagic myositis has been described in patients with a recent history of vaccination. In those cases, aluminum was identified in macrophages and thought to be derived from an adjuvant in the vaccine. In our patient there is no history of vaccination and no aluminum was identified, thus excluding that etiology, and suggesting an alternate, yet unknown, mechanism for the induction of this macrophage reaction.

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SEVERE CEREBRAL MICROANGIOPATHY ASSOCIATED WITH POSTERIOR LEUKOENCEPHALOPATHY SYNDROME (PRES): A CASE WITH PATHOGENIC IMPLICATIONS. Meenakshi Gupta, Jacqueline M. Garonzik, Sonal Hazariwala, Randolph A. Hennigar, Stephen B. Hunter and Daniel J. Brat*. Emory University School of Medicine, Atlanta, GA.

Central neurotoxicity may complicate the use of the immunosuppressant cyclosporine and is characterized clinically in a subset of patients as a posterior reversible leukoencephalopathy syndrome (PRES), with symptoms of cortical blindness, tremors, seizures, headache and altered mental status. Magnetic resonance imaging (MRI) is classic: bilateral hyperintensity on T-2 weighted images is seen in parietal and occipital lobe white matter. PRES may result from the direct toxicity of cyclosporine on the CNS or secondarily from severe hypertension following cyclosporine nephrotoxicity. We present the clinical, radiologic, and pathologic findings of PRES in a case that may suggest a central microangiopathic etiology. A 44-year-old female was treated with cyclosporine to prevent graft versus host disease (GVHD) following bone marrow transplant for myelodyplastic syndrome. Seven months later, she developed neurological complications including cortical blindness and seizures, as well as severe refractory hypertension. MRI demonstrated diffuse white matter signal abnormalities posteriorly, typical of PRES. The patient's disease was aggressive and she died 2 weeks later. Post mortem brain showed marked white matter edema, myelin pallor and astrocytosis in parietal and occipital lobes. In these same regions, a severe microangiopathy was noted, with endothelial swelling and a striking subendothelial deposition of an eosinophilic amorphous material confirmed to be fibrin on special stains. Complete destruction of the endothelial basement membrane was identified in occasional microangiopathic vessels. This vascular pathology in the distribution of the posterior leukoencephalopathy shared some histological features with that seen in cyclosporine-associated nephropathy and could suggest a role for microangiopathy in cyclosporine neurotoxicity.

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NEUROPATHOLOGIC FINDINGS IN FABRY'S DISEASE PATIENT AFTER ENZYME REPLACEMENT THERAPY WITH AGALSIDASE BETA. Amy Rapckwicz[1], Mones Abu-Asab[1], Maria Tsokos[1], Raphael Schiffman[2] and Martha Quezado[1]*. [1]National Cancer Institute/NIH, Bethesda, MD. [2]National Institute of Neurological Disorders and Stroke/NIH, Bethesda, MD. *Sponsor.

Fabry's disease is an X-linked recessive lysosomal storage disease caused by a deficiency of alpha-galactosidase A. The gene responsible for this disorder has been mapped to short arm of the X chromosome at q22.1. Characteristically, glycosphingolipids progressively accumulate in various cells throughout the body including epithelium, endothelial, ganglionic, neuronal, cardiac and smooth muscle cells. The accumulation of globotriaosylceramide, the glycolipid substrate for alpha-galactosidase A, in the intimal and medial layers of vascular endothelium is a major source of morbidity and mortality for patients afflicted with Fabry's disease. An increased incidence of early onset stroke has been found in Fabry's patients. Ischemia and infarction result from the glycosphingolipid accumulations in vascular endothelium and smooth muscle cells in both small and large cerebral vessels. This is a case presentation of the neuropathologic findings at autopsy of a 47 year old male with Fabry's disease who received enzyme replacement therapy with agalsidase beta for over 2 years. In this patient, there was diffuse cerebrovascular glycosphingolipid accumulation. Cytoplasmic accumulations of glycosphingolipids were found in smooth muscle and pericytes of cerebral blood vessels, significantly in the middle cerebral artery (MCA) distribution. Vascular endothelial cells were relatively free of deposits. Microscopic evidence of an ischemic infarction was found in the MCA distribution. In addition to vascular accumulations, glycosphingolipid accumulations were also demonstrated in leptomeningeal, neurons and paraspinal ganglia. Ultrastructural studies were performed on this material and showed parallel lipid lamellae consistent with the cytoplasmic inclusions found in Fabry's disease.

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EXTENSIVE CALCIFICATION IN A CEREBRAL BIOPSY OF A PATIENT WITH HYPOPARATHYROIDISM. Wen-Lang Lin, Elizabeth A. Shuster, Hector Robles, Murli Krishna, Robert E. Wharen Jr. and Dennis W. Dickson. Mayo Clinic, Jacksonville, FL. (Sponsored by Steven G. Younkin*.)

Background: Although cerebral calcification is a well-known complication of hypoparathyroidism, there are no reports on its ultrastructural appearance. **Objectives:** To describe the ultrastructural features of calcification in a biopsy of cerebral white matter from a 35-year-old woman with neurologic and psychiatric manifestations of hypoparathyroidism and diffuse, symmetrical subcortical white matter hyperintensity on MRI images. **Methods:** A portion of cerebral biopsy was placed in Trump's fixative and processed for routine electron microscopy (EM). **Results:** The cortex and superficial white matter had extensive vascular calcification affecting small capillaries in gray matter and venules in the white matter. Numerous calcospherites and larger calcific deposits were associated with collagenosis of vessels as shown on trichrome and a collagen immunostain. At the EM level, most endothelial cells were hypertrophic with increased number of vesicles, vacuoles and cytoplasmic organelles, but thinning and occasional breaks of the endothelium also occurred. Endothelial tight junctions were intact. The basal lamina was split, and the resulting space was filled with banded collagen fibers. Extracellular mineralized nodules and plate-like apatite crystals were present in the basal lamina. Many nodules were present in extracellular spaces between vascular and neural elements. The mineralized deposits were not associated with degenerating or necrotic cellular elements, and intracellular matrix vesicles were not detected. Inflammatory cells, limited to a few lymphocytes, and reactive changes in glia were minimal. **Conclusions:** Cerebral mineralization in hypoparathyroidism was extracellular and associated with collagen and mesenchymal cellular elements. It resembled mineralization during bone formation, rather than dystrophic calcification commonly associated with neuropathology.

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PRIMARY INTRACRANIAL HEMORRHAGE AND DRUG ABUSE: AN AUTOPSY STUDY. Iren Horkayne-Szakaly[1], Elisabeth Rushing[1]*, David Fowler[2], Miguel Riudavets[3], Juan Troncoso[3] and Ana Rubio[2]. [1]Department of Neuropathology and Ophthalmic Pathology, Armed Forces Institute of Pathology, Washington, D.C. [2]Office of Chief Medical Examiner State of Maryland; and [3]Neuropathology, Johns Hopkins University School of Medicine, Baltimore, MD.

Objectives: To investigate the frequency of drug/substance abuse in cases of fatal primary (non-traumatic) intracranial hemorrhage. **Design:** We reviewed the records of 114 cases of non-traumatic intracranial hemorrhage from the Maryland Medical Examiner's Office, 1999-2004. **Results:** 114 cases comprised 61 males (median age 51 years) and 53 females (median age 52 years). Fifty-six (50%; 30 male and 26 female) were African-American, 51 Caucasian (45%; 28 M and 23 F) and 5% other. In 65% the hemorrhage was intraparenchymal (IPH) and in 35% subarachnoid (SAH). A ruptured aneurysm was identified in 82% of SAH. The median age for SAH was 47 yrs. and 52 yrs for IPH. 35% tested positive for illicit drugs, 50% in IPH and 23% in SAH. Cocaine/ metabolites were found in 73% of drug positive cases. At the autopsy systemic atherosclerosis was found in 42% of IPH and 45% of SAH. There was no significant difference in these autopsy findings data between the drug positive and drug negative cases. Hypertensive disease was noted in 39% of IPH, and in 28% of SAH. **Conclusions:** We found that 50% of fatal cases of IPH and 23% of SAH are associated with drug use, most frequently cocaine immediately before death. We speculate that the acute hypertension caused by cocaine use contributed to the rupture of intraparenchymal vessels or aneurysm. The coexistence of drug abuse and atherosclerotic disease in our cases suggests that the pathophysiology of ICH represents a complex interplay of predisposing factors.

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VERTEBRAL ARTERY THROMBOSIS DIAGNOSED 3 MONTHS AFTER DEATH AT EXHUMATION AUTOPSY. Kathy L. Newell*. University of Kansas Medical Center, Kansas City, KS.

Vertebral thrombosis is a potentially fatal complication of neck injury that may occur in the setting of motor vehicle accidents and other trauma. Twisting of the neck vasculature may lead to endothelial injury and subsequent thrombosis although symptoms may be delayed for hours to days. A previously healthy 22 year-old man was found unresponsive at home. A CT scan revealed diffuse brain swelling and basilar hyperdensity suggestive of subarachnoid hemorrhage or vascular thrombosis. His serum contained small quantities of acetaminophen, salicylate, alcohol, and opiates were qualitatively detected. A minor car accident one day earlier caused no known injury and was considered unrelated. He died 48 hours following hospitalization. A complete autopsy confirmed diffuse brain edema and pulmonary congestion. Manner of death was interpreted as suicide from drug toxicity. Three months later, the body was exhumed and a second autopsy was performed at the University of Kansas Medical Center. Exploration of the posterior neck revealed hemorrhage in the right suboccipital triangle, but no arterial dissection was identified. Although the brain had been previously sectioned, the swollen medulla was intact, and the attached right vertebral artery contained a recent organizing thrombus. Subsequent to this finding, death was reinterpreted as an accident. Because vertebral artery thrombosis may occur following minor trauma and be associated with an asymptomatic period, the diagnosis may go clinically undetected, sometimes resulting in death. At autopsy, attention to the cerebral vasculature is particularly important with history of any recent trauma, for proper diagnosis as well as for legal implications.

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NEUROPATHOLOGY OF CHILDHOOD HOMICIDES IN THE STATE OF MARYLAND: 1994-2005. Ana Rubio[1], Miguel A. Riudavets[2], Ling Li[1], Christopher Cox[3], David Reisz[1], Iren Horkayne-Szakaly[4], David R. Fowler[1] and Juan C. Troncoso[2]. [1]State of Maryland Medical Examiner Office; [2]Department of Pathology (Neuropathology), Johns Hopkins School of Medicine; and [3]Johns Hopkins University, Bloomberg School of Public Health, Baltimore, MD. [4]Department of Neuropathology and Ophthalmic Pathology, Armed Forces Institute of Pathology, Washington, D.C. (Sponsor: Ana Rubio).

Background: The State of Maryland (population 6 million, half rural) has a unified medical examiner system that investigates suspicious deaths following standard protocols. **Method:** Cases were reviewed and tabulated for demographic characteristics, cause of death, post-injury survival, systemic and brain injuries. Cases with a significant central nervous system component were examined by a single neuropathologist (JCT). **Results:** From 1994 to 2005 one hundred and eighty five children younger than 13 years of age suffered homicidal deaths (7.5 % of all deaths reported to our office for that age group). Blunt force injuries were the most common cause of death (90 cases, 48.6%) followed by firearms (16.7%) and asphyxia (16.2%), each preferentially affecting children of specific ages. Boys were slightly more affected than girls (54.6 vs. 45.4%) and African-Americans represented 64.9% of the total. Seventy one percent of children with blunt force injuries had significant neuropathology. The findings depended on age, survival after injury and mechanism of force. Injuries included intracranial subarachnoid hemorrhage (57%), subdural (48%), hypoxic injuries (35.5%), cortical contusions (34%), swelling (21%), epidural hemorrhage (14%) and gliding contusions (12%). White matter tears and diffuse axonal injuries were rare. Intraspinal hemorrhage was seen in 31% (subdural 18%, subarachnoid 17% and epidural 12%). **Conclusion:** Brain pathology is a common finding in childhood homicide, especially in cases with a blunt force component. Detailed, systematic study and documentation of the central nervous system injuries is essential in determining the nature and timing of the injuries and ruling out natural diseases or accidental injuries.

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ASTROCYTE ATYPIA ASSOCIATED WITH GAMMA KNIFE AND WHOLE BRAIN RADIATION THERAPY. Howard T. Chang* and Robert L. Schelper*. SUNY-Upstate Medical University, Syracuse, NY.

Whole brain radiation therapy and gamma knife radiosurgery have been used to treat both primary and metastatic tumors in the brain. Since imaging studies often cannot distinguish unequivocally recurrent tumor from radiation necrosis, neuropathologists are often asked to evaluate whether the biopsy of abnormal tissue contain recurrent tumor or radiation therapy related reactive changes. One of the more vexing problems is to distinguish reactive astrocytes with nuclear and/or cytological atypia from recurrent glioma cells. To address this issue, brain tissue with reactive and/or atypical astrocytes from several groups of patients are examined. These patients include those with either glioma or metastatic tumors (e.g., carcinoma) status post radiation therapy (most patients in these groups have received both gamma knife and whole brain irradiation). The third group consists of patients with non-neoplastic brain lesions (e.g., strokes, demyelinating or inflammatory lesions) and without history of radiation exposure. Micrographs of representative "reactive" or "atypical" astrocytes from each of these groups are compared with each other. Our preliminary observations show that: 1) reactive astrocytes can have considerable nuclear atypia even without radiation therapy; 2) cells with marked nuclear atypia but without abundant pink cytoplasm are likely recurrent/residual neoplastic glioma cells; and 3) unless gemistocytic astrocytoma cells were found within the original glioma, singly astrocytes with abundant pink cytoplasm, with or without nuclear atypia, are probably "reactive" rather than neoplastic.

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IMMUNOHISTOCHEMICAL EXPRESSION OF TAU PROTEIN KINASES IN GANGLIOGLIOMA WITH NEUROFIBRILLARY TANGLES. Hajime Miyata, Yoshie Matsuo, Masamichi Kurosaki, Hideki Kamitani and Eisaku Ohama. Institute of Neurological Sciences, Faculty of Medicine, Tottori University, Japan. (Sponsored by Harry V. Vinters*.)

We report an immunohistochemical study of ganglioglioma, grade II, with neurofibrillary tangles (NFTs) in a 47-year-old Japanese woman presenting with longstanding intractable epilepsy, to clarify the mechanism of tau protein phosphorylation within the dysplastic neurons. Formalin-fixed, paraffin-embedded biopsy specimens were subjected to immunohistochemical study. Immunostaining was performed by the Labeled Polymer method with 3,3'-diaminobenzidine tetrahydrochloride as chromogen. The tumor consisted of GFAP-positive neoplastic astrocytes mixed with abundant small-sized dysplastic neurons. There were no mitotic figures or area of necrosis; the MIB-1 labeling index was less than 0.1%. Neuronal cells within the tumor were immunoreactive for CD34 class II antigen, and some were also positive for nestin. Many neuronal cells contained globose-type NFTs that were positive for AT8, 3 repeat (R)-tau, 4R-tau, PHF, and phospho-JNK; some of them were also positive for phospho-Cdk5 (Ser 159) and ubiquitin. Phospho-p44/42 MAPK and GSK3β (Ser9) were detected in the cytoplasm of NFT-bearing neurons but not within the NFTs. Phospho-JNK expression was observed both in the cytoplasm and NFT. Immunoreactivity for phospho-p38, α-synuclein, and Aβ protein was not detected. Neither NFT nor senile plaques were observed in the relatively normal cerebral cortex adjacent to the tumor. These results suggest a mechanism of tau phosphorylation distinct from that of Alzheimer disease.

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PRIMARY LEPTOMENINGEAL OLIGODENDROGLIOMA WITH CHROMOSOME 1p DELETION: A CASE REPORT AND REVIEW OF THE LITERATURE. T. David. Bourne, James W. Mandell*, Julie A. Matsumoto and M. Beatriz S. Lopes*. University of Virginia School of Medicine, Charlottesville, VA.

We report the case of a two-year-old male with a primary, diffuse leptomeningeal oligodendroglioma which showed deletion of chromosome 1p by fluorescence in situ hybridization (FISH). This previously healthy infant initially presented with malaise, anorexia, nausea, vomiting, and macrocephaly. Imaging studies confirmed the presence of hydrocephalus, so a ventriculoperitoneal shunt was placed. The postoperative course was complicated by emesis, continued weight loss, and numerous seizure-like episodes. An MRI with contrast showed diffuse leptomeningeal thickening and enhancement without evidence of an intraparenchymal mass lesion. A right frontal lobe brain biopsy showed a hypercellular proliferation of small oligodendroglioma-like cells (OLC) which diffusely involved the leptomeninges and spared the underlying cortical gray matter. The tumor cells showed prominent perinuclear clearing and had evenly spaced, uniformly round nuclei. Occasional mitotic figures were observed. Background vessels were thin and delicate and there was no evidence of necrosis. The tumor cells showed strong immunoreactivity for S-100, and were negative for GFAP, vimentin, EMA, Neu-N, and synaptophysin. FISH for analysis of chromosomes 1p and 19q was performed on paraffin embedded tissue and showed deletion of chromosome 1p. Chromosomes 1q, 19p, and 19q were intact. To our knowledge, this is the first reported case of a 1p chromosomal deletion in primary diffuse leptomeningeal oligodendroglioma.

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DYNAMIC GENOMIC DELETION EXPANSION: A MOLECULAR MARKER OF HIGH GRADE GLIOMAS. S. D. Finkelstein, P. A. Swalsky and M. M. Wilson. RedPath Integrated Pathology, Pittsburgh, PA.

Background: Defining the presence of anaplastic transformation in gliomas can be challenging yet critical to accurate diagnosis. Morphologic features are important as changes can be focal. Molecular parameters such as mutational load may be helpful. Here we report a novel observation pertaining to genomic stability useful when deciding on anaplastic transformation. **Methods:** 8 low grade and 9 high grade gliomas were microdissected at four or more locations. Targets were based on cellular heterogeneity and included peripheral tumor infiltration. Each target was evaluated for allelic imbalance using a broad panel of microsatellite markers (1p,5q,9p,10q,17p) situated near tumor suppressor genes. Additional markers for imbalanced genomic region were used to define deletion stability. All specimens were approved and de-identified for this study. **Results:** Most high grade gliomas (6/8, 75%) demonstrated an increasing gradient of genomic deletion expansion towards the tumor periphery. In contrast, all low grade glioma manifested no such a change ($p<.01$) maintaining deletion stability when present. In 4 cases where high grade gliomas possessed distinct low grade components (precursors for anaplastic transformation) the shortest region of genomic deletion was present in the low grade area. Glioma cells at the infiltrating edge had the widest genomic deletion. **Conclusions:** Genomic deletion, the second of two steps in tumor suppressor gene inactivation, can become unstable and progressively widen in high grade, anaplastic glioma biology. Such changes could impact phenotypic expression and be useful in glioma diagnosis and prognostication.

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THE METHYLOME IN GLIOBLASTOMA. Wolf C. Mueller, Markus J. Riemenschneider, Catherine L. Nutt and David N. Louis. Department of Pathology, Cancer Center and Neurosurgical Service, Massachusetts General Hospital and Harvard Medical School, Boston, MA.

Pharmacologic reversal of epigenetic silencing by 5́Aza-2-deoxycytidine (5́Aza-dC) combined with expression profiling is a powerful tool for comprehensive analysis of methylation-related gene regulation and, possibly, for the identification of tumor-related genes. Triplicates of three primary short term glioblastoma cultures were exposed to 5μM 5-aza-2-deoxycytidine (5-Aza-dC) for 96 hours followed by cRNA hybridization to an oligonucleotide microarray chip (Affymetrix U133A chip; 22,284 probe sets). Probe sets were selected for an average raw expression of greater than 20 in the treated samples and a greater than three fold increase in expression following 5́Aza-dC treatment; 110 probe sets met these criteria. Fifty of these probe sets, representing 42 genes, were statistically significant at $p < 0.05$. Affected chromosomal regions included areas frequently lost in glioblastoma, indicating genes with potential tumor suppressor function. Bioinformatics revealed CpG islands in the promoter region of 17 of these 42 genes. Ongoing candidate validation on glioblastoma cultures revealed promoter hypermethylation in all of the genes studied to date, demonstrating the strength of the method. In addition to cell-type specifically methylated genes and genes potentially involved in early brain development, preliminary data indicate two novel candidate genes epigenetically regulated in glioblastoma tumorigenesis.

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METHYLATION OF THE PROMOTER OF TP53 GENE IN GLIOBASTOMA. Sung-Hye Park, Yu-Jung Lee and Bo-Mi Kim. Department of Pathology, Seoul National University College of Medicine, Seoul, Republic of Korea (Sponsored by Harry V. Vinters).

Object: TP53 is a central tumor suppressor gene in humans and most tumor suppressor genes can be silenced by promoter methylation. Glioblastoma have functional inactivation of the TP53 pathway but TP53 mutations are less frequent than TP53 overexpression. The object of this study is to find the promotor methylation status of TP53 in glioblastoma (GBM). As far as we know, in glioblastomas, none of published data of the promotor methylation of TP53 exist. **Methods:** p53 protein expression was evaluated on tissue microarrays of 19 GBMs by immunohistochemistry. Methylation specific PCR (MSP), cloning and sequencing of this MSP products with extracted genomic tumor DNA from paraffin blocks. Direct sequencing of the exon 5, 6, 7 and 8 of TP53 were performed in all cases to see the mutation. **Results:** p53 protein overexpression was found in 10 (52.5%) cases. Direct sequencing of exon 5,6,7,and 8 resulted in mutation in 2 cases (20%). TP53 promotor was methylated in 8 (42%) cases, all of which were confirmed by cloning and sequencing of these MSP products in over 80% of CpG sites, and which had no TP53 gene mutation. Five (62.5%) out of eight TP53 promotor methylated cases revealed no expression of p53 protein in immunohistochemistry, suggest silencing of TP53 by promotor methylation. **Conclusion:** Silenicing of p53 tumor suppressor gene by promotor methylation may be the important alternative mechanism of tumorigenesis of the GBMs as well as overexpression of abnormal p53 protein by mutation or other mechanisms.

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ASTROCYTOMA OF THE THORACIC SPINAL CORD WITH CLEAR CELL COMPONENT AND DISSEMINATION–REPORT OF A CASE WITH IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL STUDY. Zhongxin Yu[1], Rhonda Shuey-Drake[1], Kalliopi Petropoulou[2], Johnnie Honeycutt[3] and Kar-Ming Fung[1]. [1]Department of Pathology, [2]Department of Radiology, and [3]Department of Neurosurgery, University of Oklahoma Health Science Center, Oklahoma City, OK.

A thirteen-year-old boy presented to our institution with progressive lower extremity weakness, back pain, scoliosis and incontinence. Magnetic resonance imaging of the spine revealed an intramedullary mass with solid and cystic components that expand the thoracic spinal cord and thecal sac at the level of T6 through T10. The mass was isointense to hyperintense to the white matter on T1-weighted images with heterogenous enhancement. There was also abnormal enhancement along the posterior surface of the spinal cord at the levels of T1 and T3 and the cauda equina consistent with leptomeningeal dissemination. The patient underwent a T6 through T12 laminoplasty with excision of the tumor. Pathology examination revealed a low-grade astrocytic neoplasm with clear cell component that morphologically resembled neurocytoma or oligodendroglioma. The clear cells were weakly immunoreactive for synaptophysin when a polyclonal antibody was used but nonreactive when a monoclonal antibody (SY38) was used. On ultrastructural studies of the clear cells, there were no dense core (neurosecretory) granules or other features that would suggest neuroendocrine or neuronal differentiation. We concluded that the clear cell component did not have neuronal or neuroendocrine differentiation. The clinical-pathologic features of our case are similar to the recently described spinal low-grade glial tumor with leptomeningeal dissemination. Clear cells have also been described in some of these tumors and neuroendocrine differentiation has been identified in the clear cells by immunohistochemistry. Our study suggests that ultrastructural study is an important diagnostic adjunct when the neuroendocrine differentiation is questionable by immunohistochemistry.

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GLIOBLASTOMA WITH REMOTE SUBCUTANEOUS METASTASES. Lisa L. Pate, Terri L. Haddix and Hannes Vogel. Stanford University Medical Center, Stanford, CA.

Systemic metastases from primary central nervous system neoplasms are rare. Prior surgery or the presence of a shunt has been associated with an increased risk of extracranial metastases. There are scattered case reports of metastases from glioblastoma (GBM) and rare reports of subcutaneous metastases, most likely representing intraoperative seeding of the scalp. We report the case of a 50-year-old gentleman who presented with a history of forgetfulness, expressive aphasia and headache. Imaging showed a 4.4 × 3.5 cm ring enhancing mass in the anterior left temporal lobe associated with significant edema. The histology of the resected mass demonstrated findings typical of GBM. Seven months later, after radiation and chemotherapies, he presented with left facial pain and diplopia. Two subcutaneous nodules were incidentally noted on his back. A large intracranial and infratemporal tumor recurrence was found on imaging. A second extensive resection was performed; the two subcutaneous masses were also excised. All portions of the second specimen showed a tumor with only adenoid and spindled growth patterns, which were not found in the original resection. Given the markedly different histologic appearance of the second resection specimen, immunoperoxidase studies were pursued. The tumor cells, irrespective of phenotype, demonstrated uniform and strong immunopositivity for S-100 and GFAP only, concordant with the diagnosis of metastatic GBM. This case represents an example of the rare entity of GBM with remote subcutaneous metastases. As the second resection specimen bore no histologic resemblance to the original tumor, other secondary neoplasms (including carcinomas) were considered and excluded.

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OVEREXPRESSION OF OSTEOPONTIN (OPN) IN GLIOBLASTOMA MULTIFORME (GBM). Terri L. Haddix, Lawrence D. Recht, Timothy Myles, Lawrence LK. Leung and Hannes Vogel. Stanford University Medical Center, Stanford, CA.

In addition to bone remodeling, OPN is frequently overexpressed in metastatic cancers, suggesting an important role in tumor aggressiveness. Although OPN gene upregulation has been reported in high grade gliomas, there has been no systematic study of this glycoprotein in brain tumors. We used three antibodies, a commercially available murine monoclonal (mAb) against a rodent bone fraction that reportedly identifies human OPN and two rabbit polyclonal antibodies from our laboratories against synthetic human OPN peptides around the thrombin cleavage site (full-length (FL) and thrombin-cleaved lacking the terminal arginine (TCA), to assess OPN expression in formalin-fixed paraffin sections of a variety of tumor types and normal brain tissue. Virtually no OPN staining was seen in any brain section, including GBMs, using mAb. Immunopositivity for FL or TCA antibodies was found in all tested gliomas, but was most reliably found in GBMs. In the additional 13 GBMs, immunopositivity for FL or TCA antibodies was found in 12 and 13 cases, respectively. Interestingly, the most intense staining was seen in a patient with GBM metastatic to extracranial sites. Thus, similar to reports of other tumors, OPN expression correlates with glioma aggressivity. The differences in staining between the monoclonal and polyclonal antibodies suggest that this relationship may have been underestimated in the past. Since OPN is known to be a ligand for both CD44 and $\alpha v\beta$ integrins, both important mediators of glioma invasiveness and angiogenesis, further work is warranted to assess the role of OPN as a potential marker or motor of glioma aggressivity.

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PEDIATRIC CEREBELLAR OLIGODENDROGLIOMA: REPORT OF A RARE CASE AND REVIEW OF THE LITERATURE. Sejal Shah, Robert L. Schelper* and Howard T. Chang*. SUNY-Upstate Medical University, Syracuse, NY.

We report a rare case of cerebellar oligodendroglioma in an 11-year-old boy: The patient fell from a tree and MRI of the brain revealed a 2 cm mass in the left lateral cerebellum. Prior to this accident the patient was in a good state of health and neurological examination revealed no focal deficits. The mass was hypo-intense on T1 and hyper-intense on T2 weighted images, respectively. No enhancement was seen with intravenous contrast material. There was no evidence of intraparenchymal hemorrhage or midline shift. The radiological impression was that of a low grade glioma. Microscopic examination of the excised tumor showed neoplastic glial cells with small round or oval nuclei and scant pale perinuclear cytoplasm. The tumor cells infiltrate the cerebellar cortex and involve the leptomeninges. No Rosenthal fibers or eosinophilic granular bodies are seen. Immunocytochemistry shows that the tumor cells are weakly positive for S-100, and negative for GFAP, synaptophysin and Neu-N. A low mitotic activity is reflected in a low Ki-67 labeling index. These features are consistent with an oligodendroglioma. Most oligodendrogliomas arise in adults and are located in the cerebral hemispheres. Less than 10 cases of pediatric cerebellar oligodendroglioma have been reported, and most of these had good prognosis after therapy. Our patient, after gross total resection, showed no evidence of tumor recurrence at three months follow-up.

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MICROVASCULAR MORPHOLOGY AND IMMUNOLOCALIZATION OF BLOOD BRAIN BARRIER MARKERS AND VEGF DURING EXPERIMENTAL GLIOMA DEVELOPMENT. José V. Lafuente*, Susana Bulnes, Harkaitz Bengoetxea and Enrike Argandoña. University of Basque Country, Leioa, Spain.

During brain tumours growth pre-existing blood vessels adapt their morphology and increase vascular permeability expressing the proangiogenic vascular endothelial growth factor (VEGF). We report a study of the microvascular morphology, BBB expression markers and VEGF at different stages of development. Glial tumours were induced in rats by transplacentary administration of Ethylnitrosourea (ENU) on the 15th pregnant day. Tumours were localized by RMI and by Evans Blue dye at autopsy time. Conventional histological and immunohistochemical techniques were performed for Haematoxylin Eosin (HE) and LEA lectin histochemistry, GluT-1(Glucose transporter -1, Chemicon AB1340, 1:1000), EBA (Endothelial barrier antigen, Sternberger SMI 71, 1:1000) and VEGF (A-20 Santa Cruz 1:75). All animal experiments were performed in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC). Histopathological patterns by HE and Ki-67 labelling Index permit to classify the tumours in four development stages. Tumour stages I-II shown cerebral parenchyma similar vessels. In contrast, at stages III-IV blood vessels became elongated, tortuous, aberrant and with an increased diameter. Immunopositivity for GluT-1 and EBA was observed on vascular wall in early development stages (I, II and III) whereas in the IV there was a lack of EBA expression. At this time GluT-1 immunopositivity still remained in some vascular sections co-expressing with VEGF. Microvessels at advanced stages became dilated and with increased permeability corresponding to overexpression of VEGF. The BBB structure function was lost whereas the metabolic function persisted in vascular sections. **Acknowledgements:** This work was supported by a Basque Government predoctoral grant.

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HIGH PROLIFERATIVE ACTIVITY IN ANAPLASTIC PLEOMORPHIC XANTHOASTROCYTOMA ASSOCIATED WITH MULTIPLE RECURRENCES IN A 2-YEAR PERIOD. Nketi Forbang, Kathy L. Newell* and Steven B. Wilkinson. University of Kansas Medical Center, Kansas City, KS.

Pleomorphic xanthoastrocytoma (PXA), usually a WHO grade II tumor, tends to follow a relatively benign clinical pathway with long survival and cures possible following surgical resection. Histological criteria for anaplastic PXA are not well defined. Herein, we report on a PXA with anaplastic features and aggressive clinical behavior throughout its course. A 19 year-old previously healthy man presented with headache, left sided weakness, and a right parietal intraparenchymal hemorrhage. Tumor was discovered at the time of the hematoma evacuation. The histological findings showed a mitotically active astrocytic tumor with pleomorphic cells and reticulin staining, but no foamy cells or eosinophilic granular bodies were recognized. The MIB-1 labeling index was 54%. Following surgery, radiation and chemotherapy were administered and serial magnetic resonance imaging was used for surveillance. Tumor recurrences occurred at 17, 19, 20, and 22 months after initial presentation. In each recurrence, the histological findings included abundant eosinophilic granular bodies, reticulin rich areas, xanthomatous cells, frequent mitoses, and areas of necrosis. Pleomorphic cells were sparse. Immunoreactivity to glial fibrillary acidic protein and synaptophysin antibodies was present in areas. In the final postoperative period, the patient developed acute respiratory distress with multiple pulmonary emboli diagnosed radiographically. His clinical condition deteriorated and death occurred nearly 23 months following initial presentation. A brain only autopsy confirmed residual tumor confined to the dura. Although histological criteria for anaplastic PXA are still unsettled, high proliferative activity appears to have been an accurate predictor of the aggressive clinical course in this case.

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1p/19q CHROMOSOMAL DELETIONS IN METASTATIC OLIGODENDROGLIOMA. Cheryl Ann. Palmer[1]*, Ryan Merrell[1], L. Burton. Nabors[1] and Arie Perry[2]. [1]University of Alabama at Birmingham, Birmingham, AL; and [2]Washington University, St. Louis, MO.

Extracranial metastasis of primary brain tumors is a rare phenomenon. Occasional cases have been reported of metastatic oligodendroglioma, but only a few have evaluated their genetic composition, specifically searching for deletions of chromosomes 1p and 19q. We report two patients with metastatic oligodendroglioma to bone who sustained long-term survivals. Neuropathologic evaluation and genetic analysis were performed on their tumors. Although the bone specimen was non-informative in one patient, the tumor in one of these patients revealed relative deletions in both chromosomes 1p and 19q, recognized as a genetically favorable profile. As we learn more about the chemotherapeutic responsiveness of anaplastic oligodendrogliomas associated with deletions of chromosomes 1p and 19q, patient survival may continue to increase long enough to permit the more frequent emergence of metastatic gliomas.

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MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) TO DETECT LOSS OF CHROMOSOME 1P AND 19Q IN OLIGODENDROGLIAL TUMORS: SETTING THE GROUNDRULES. Judith W.M. Jeuken, Sandra J.B. Cornelissen, Sandra H.E. Boots-Sprenger and Pieter Wesseling*. Radboud University Medical Centre, Nijmegen, The Netherlands.

An accurate distinction between oligodendroglial tumors (OTs) and astrocytic tumors is very important because of the prolonged survival and chemosensitivity of (part of) the OTs. Loss of 1p (-1p) in OTs is usually associated with -19q and has repeatedly been reported as a positive predictor for chemosensitivity and prolonged survival. As the responsible genes on 1p/19q have not yet been identified and patients with a complete loss show a better survival than those with a partial loss, analysis of multiple regions on 1p/19q is preferable. Using MLPA (multiplex ligation-dependent probe amplification) DNA copy number changes of approximately 40 loci can be detected in a relative simple single semi-quantitative PCR-based experiment. Therefore the easy to use MLPA technique is more suitable than the often used LOH or ISH analysis. Using our set of (oligodendro)glial tumors that were genetically characterized by CGH, we evaluated a commercially available "1p/19q oligodendroglioma MLPA kit". Major pitfalls include the DNA isolation protocol and the amount of DNA used. Furthermore, in this MLPA kit control probes to monitor reaction efficiency are included however no strict guidelines are provided. Our experiments show that very strict criteria are essential to avoid false positive identification of loss of 1p/19q, unreliable probes were identified and criteria for the identification of 1p/19q tumors were established. We conclude that so far a reliable distinction between gliomas with or without -1p/-19q by MLPA can only be made after application of stringent quality control criteria and using high quality and amount of DNA.

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ANAPLASTIC PROGRESSION OF PILOCYTIC ASTROCYTOMA OF THE MEDULLA. Irina Mikolaenko, Christian Roth, Shetty Rajit, Susan Chi, R. Michael. Scott and Rebecca D. Folkerth*. Children's Hospital, Brigham and Women's Hospital; and Dana-Farber Cancer Institute, Boston, MA. University of Technology Dresden Medical School, Dresden, Germany.

Pilocytic astrocytomas (PA) arise in the cerebellum, optic system, or hypothalamus, and behave as WHO grade I. Rarely, they involve the cerebral hemispheres, spinal cord, or medulla (as dorsally exophytic masses). While anaplastic progression of cerebellar PA has been described, usually after irradiation, such behavior in brainstem PA has not. The patient presented at age 4 with ataxia. MR showed medullary expansion by a low-signal cystic mass, with an enhancing nodule posteriorly, confirmed on biopsy as PA. She was observed for 1 yr until progressive growth necessitated a second resection, showing the same histology; radiotherapy (5580 cGy) was given. She was asymptomatic for 10 yrs, until MR detected growth; biopsy again showed PA, and she received chemotherapy. After another 3 yrs, a 4th biopsy showed anaplastic transformation, with areas of classic PA merging with increased cellularity, pleomorphism and mitoses. She underwent additional multiagent chemotherapy. After 1 year, at age 20, the patient died with respiratory failure and sepsis. Autopsy revealed circumscribed astrocytic tumor in the medulla and dorsal cervical cord. There was multifocal transition from typical PA, with Rosenthal fibers and subarachnoid spread, to highly malignant glioma, with pseudopalisading necrosis. Despite the anaplastic histology, tumor infiltration at the margins was only seen focally in the cerebellum. While second malignancies (usually glioblastomas, meningiomas, or sarcomas) are well-known following radiation, transformation of a grade I astrocytoma to a grade IV astrocytoma, but retaining PA features, particularly outside of the cerebellum, and after such a long interval of 13 years, is very rare.

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SEMIQUANTITATIVE APPROACH TO THE VEGF IMMUNOPOSITIVITY IN HUMAN GLIOMAS AND ITS CORRELATION WITH PROLIFERATION RATE, OEDEMA SPREADING AND CLINICAL BEHAVIOUR. Alex Carrasco[1], Iñigo Pomposo[1], Jesús Maria Garibi[1] and José V. Lafuente*[2]. [1]Cruces Hospital and University of Basque Country, Baracaldo, Spain. [2]University of Basque Country, Leioa, Spain.

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific cytokine related to vascular permeability and angiogenesis. Microvessels density and VEGF level have been related to survival in low grade astrocytomas (LG). We evaluate relationships between VEGF-LI, proliferation rate, peritumoral oedema, survival and the patient's functional status according to the Karnofsky Performance Scale (KPS). Paraffin slices from 87 malignant gliomas (anaplastic astrocytomas and glioblastoma multiforme) were incubated with polyclonal antibodies to VEGF and Ki-67. Labelling indexes (LI) (% of immunopositive cells) were determined by counting 400 tumour cells. The peritumoral oedema was evaluated in 3 degrees of severity according to Kazner scale. Ki-67 LI segregates significantly ($p<0.01$) both histological groups (AA = 8.7 and GBM = 14.2), and the three subgroups of oedema (1 = 7.1; 2 = 10.9; 3 = 23.7). Immunopositivity for VEGF was observed in all specimens, in vessels wall and in tumour cells, especially around the perivascular area, LI was around 25% for both histological subtypes. Oedema subgroups were segregated but not significantly by this parameter (1 = 17; 2 = 24; 3 = 32). Regarding quality of life, preoperative and postoperative (1 month after surgery) KPS = >90 seems to be associated to low VEGF LI showing strong correlation to longer survival ($p = 0.01$)*. That is independent of the histological diagnosis, thus VEGF LI has a predictive prognostic value. **Acknowledgements:** This work has been partially supported by grant G03/114 from MSC (Spain).

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OLIGODENDROGLIAL LINEAGE MARKER EXPRESSION IN EPENDYMAL TUMORS. M. Snuderl, S. N. Chi, J. A. Chan, M. A. Rubin, M. W. Kieran and K. L. Ligon*. Brigham and Women's Hospital, Children's Hospital Boston, Dana-Farber Cancer Institute, and Harvard Medical School, Boston, MA.

Histopathologic prediction of clinical outcome for pediatric intracranial ependymomas is unreliable and few useful adjunct molecular markers have been identified. Cell cycle marker expression has been extensively analyzed while developmental lineage specific markers have been less well studied. The oligodendroglial lineage marker OLIG2 has recently been identified as being variably expressed in small numbers of ependymomas and could identify clinically distinct subgroups of patients. To better characterize OLIG2 expression and correlate with clinical outcomes, we performed an immunohistochemical study of OLIG2 expression in clinically annotated tissue microarrays of 32 pediatric intracranial ependymomas. Overall, 66% of ependymomas expressed OLIG2. Expression was more commonly seen in low grade tumors (85%, 17/20) than in high grade tumors (33%, 4/12). Expression was not consistently associated with any specific histologic patterns, including clear cell variants. OLIG2 expression was not significantly correlated with clinical outcomes such as progression free survival or overall survival. Correlation of OLIG2 status with the expression of other markers such as MIB-1, p53, cyclinD1, Bcl-2, or Topoisomerase II-alpha failed to detect any statistically significant associations. In summary, expression of the oligodendroglial marker OLIG2 is a common feature of ependymal neoplasms raising questions about the cellular origins and makeup of these tumors, but does not reliably aid in predicting clinical outcome.

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TRANSGENIC MICE OVEREXPRESSING PLATELET DERIVED GROWTH FACTOR B (PDGF-B) DEVELOP SCHWANNOSIS AND ASTROCYTOMAS: A POSSIBLE ANIMAL MODEL FOR NEUROFIBROMATOSIS. Yasuyuki Hitoshi, Mark A. Israel and Brent T. Harris*. Dartmouth Medical School, Hanover, NH.

Neurofibromatosis (NF) type1 and type2 are genetic disorders with distinguishing clinical and pathological features. Both disorders are characterized by glial and Schwann cell proliferations. Generally these are benign proliferations that manifest as neurofibromas or Schwannomas, although malignant tumors such as fibrosarcomas and astrocytomas can occur. Previous studies have suggested a role for PDGF signaling in the proliferation of Schwann cells derived from patients with NF. To explore further the role of PDGF-B in the pathogenesis of NF, we used the human GFAP promoter to generate transgenic mice expressing PDGF-B. These animals express high levels of PDGF-B in their spinal cord, where diminished spinal white matter tracts and disorganized spinal cord grey matter were observed. These mice also develop Schwannosis, a benign proliferation of Schwann cells indistinguishable from the Schwannosis seen in NF2 patients and transgenic mice expressing a dominant negative NF2 protein (merlin). Schwannosis in our mice is characterized by uniform spindle-shaped cells found surrounding the spinal nerve roots and in subarachnoid collections. These cells also infiltrate the spinal cord and immunostain strongly for S100 and occasionally for GFAP. When our GFAP-PDGF-B mice were crossed into a $P53^{+/-}$ background, the resultant mice occasionally developed astrocytomas. These animals show cataracts frequently and epilepsy and hydrocephalus occasionally, features sometimes seen in NF. These findings provide strong evidence that PDGF-B is an important mediator of multiple different features of the syndrome that constitutes neurofibromatosis and is consistent with recent findings implicating the PDGF receptor in cellular pathological changes mediated by merlin.

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IN SITU ANALYSIS OF THE INTEGRIN SIGNALING PATHWAY IN HUMAN GLIOBLASTOMAS. Markus J. Riemenschneider[1], Wolf Mueller[1], Rebecca A. Betensky[2], Gayatry Mohapatra[1] and David N. Louis*[1]. [1]Massachusetts General Hospital and Harvard Medical School; and [2]Harvard School of Public Health, Boston, MA.

Deregulated signaling via integrins is common in many types of cancers, including glioblastoma. Integrin binding as well as growth factor receptor (GFR) signaling results in activation of focal adhesion kinase (FAK) and subsequent upregulation of the extracellular regulated kinases (ERK-1/2), leading to cell cycle progression and cell migration. Most studies of this pathway have used *in vitro* systems or tumor-lysate based approaches. We aimed to delineate this pathway in an *in vivo/in situ* manner using immunohistochemistry and fluorescence in situ hybridization (FISH) in a panel of 30 glioblastomas. Evaluating the topological distribution of molecular changes at a histological level within individual tumors, we found increased expression levels of integrins, (p-)FAK, the docking molecule paxillin (PXN) and (p-)ERK-1/2 in regions defined by elevated EGFR and/or PDGFRA expression. Moreover, the level of FAK activation showed a significant correlation with EGFR and PDGFRA expression, and p-FAK and EGFR expression colocalized on a single cell level on immunofluorescent double labeling. Integrin expression was more regionally confined than FAK, p-FAK and PXN expression with integrins $\beta 8$ and $\alpha 5\beta 1$ being the most widely expressed integrins, often showing perinecrotic or perivascular expression. In summary, our data indicate that GFR overexpression facilitates alterations in integrin signaling. In addition, overall FAK activation in glioblastoma appears related to GFR expression. Integrins may also play a role in the tumor microenvironment, enhancing the impact of GFR signaling on the expression and activation of downstream kinases in critical morphologically defined regions.

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CYTONECTIN "A DO NOT ATTACK MOLECULE" IS OVEREXPRESSED IN GLIOBLASTOMA MULTIFORME. Martha Quezado[1]*, Elsa Lin Ning. Tapia[1], Elisabeth Rushing[2], Kevin Camphausen[1] and Soni J. Anderson[1]. [1]National Cancer Institute/NIH, Bethesda, MD. [2]AFIP, Washington, D.C. *Sponsor.

Highly insoluble proteins and associated phagocytic cell foci are found in many important pathologic conditions. These include Alzheimer's disease and other tauopathies. Our recent research indicates an association of insoluble protein with macrophage mediators of pathology in solid tumors, including brain cancer. The highly insoluble protein, cytonectin, was used as a test molecule in a new protocol, reversed order O'Farrell gel electrophoresis (ROOGE). This new method is useful in the separation of proteins that do not resolve by standard purification techniques. Normal human term placental cytonectin was ROOGE-purified and employed as antigen to develop chicken anti-human cytonectin antibodies. These were used in immunoblots to demonstrate overexpression of cytonectin in cancer patient tissues. Ineffectual microglial/macrophage attack is commonly observed in glioblastoma mutltiforme, a uniformly fatal brain cancer. Based upon this observation, we predicted that cytonectin would be overexpressed in high grade gliomas. In our preliminary studies, all cases of glioblastoma multiforme (5) tested showed overexpression of cytonectin compared to matched normal brain specimens. Immunomicroscopy revealed nuclear and cytoplasmic staining. Since cytonectin is thought to act as a "do not attack" molecule that defends cells against phagocyte destruction, its overexpression in tumors may prevent immunosurveillant clearing of cancer cells. Cytonectin is therefore a likely candidate target for future molecular therapeutic applications.

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NESTIN EXPRESSION IN NEOPLASTIC AND NON-NEOPLASTIC BRAIN. Jeffrey Sosnowski, Charles G. Eberhart and Wolfram Kleeburger. Johns Hopkins University School of Medicine, Baltimore, MD.

Objective: To evaluate nestin protein expression in primary brain tumor samples. **Methods:** Immunohistochemical analysis of tissue arrays using an anti-human nestin antibody. **Results:** Nestin is an intermediate filament protein expressed in neuroepithelial stem cells. It is not usually found in mature neurons or glia. Little is known about nestin expression in primary brain neoplasms. We assessed a wide range of brain tumors for nestin immunoreactivity using tissue arrays containing 20 oligodendrogliomas, 20 grade II astrocytomas, 20 grade III anaplastic astrocytomas, 20 grade IV glioblastoma multiforme, and 80 embryonal brain tumors. Immunoreactivity for nestin was identified in all tumor types, with heavy expression in glioblastomas and medulloblastomas. However, nestin immunoreactivity was also present in both reactive astrocytes and microglial cells adjacent to neoplastic elements, suggesting this protein is not specifically expressed in tumors.

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PILOMYXOID MORPHOLOGY IN GLIONEURONAL TUMORS. S. H. Gultekin*, M. Grafe, N. Selden and A. West. Oregon Health & Science University, Portland, OR.

Objective: To investigate the prognostic implication of pilomyxoid glial component in glioneuronal tumors. **Materials & Methods:** Two patients with this morphology are compared with regards to clinical features, histopathological work-up and follow-up. **Results:** The first patient is a 77-year-old female with a 6-week history of progressive gait disorder who underwent gross-total resection of a deep cerebellar ring-enhancing tumor. Pathology revealed a ganglioglioma with a glial component of typical pilomyxoid morphology (PMM) with mitoses, a MIB-1 index of 8%, and focal microvascular proliferation (MVP). The neuronal component consisted of mature ganglion cells with binucleate forms. She has been recurrence-free for eight months. The second patient is a 12 month-old male who presented with rapidly progressive lower extremity weakness. MRI revealed an intramedullary (T1 to T5) enhancing lesion. Pathology revealed a pilomyxoid neoplasm with a similar ganglion-cell component, a MIB-1 index of 4%, and focal MVP. The tumor recurred aggressively within 3 months of the initial gross-total resection and chemotherapy, and underwent a re-excision. Histopathology showed abundant MVP, and persisting PMM. EM confirmed the glial nature of the bland pilomyxoid component in both cases, and no ependymal features were observed. **Conclusions:** 1. PMM may be observed as a component of glioneuronal tumors. 3. Pilomyxoid morphology combined with MVP and a relatively high MIB-1 index may imply different tumor behavior in different age groups. 3. PMM may herald early recurrence in glioneuronal tumors in children.

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CENTRAL NERVOUS SYSTEM NEOPLASIA IN 1990-1991 GULF WAR VETERANS. Charles S. Specht*, Michael R. Lewin-Smith, Linda A. Murakata, Elisabeth J. Rushing, Glenn D. Sandberg, Victor F. Kalasinsky, Albin L. Moroz and Florabel G. Mullick. Armed Forces Institute of Pathology (AFIP), Washington, D.C.

The AFIP Kuwait/Persian Gulf Registry contains data from 7,745 U.S. veterans of the 1990-1991 Gulf War (GWVs) who have had anatomic pathology material submitted to the AFIP. We reviewed the Registry database for CNS neoplasia; 83 patients with biopsied tumors were identified. The 46 patients with low-grade tumors were aged 22-63 years (average 38.1 years). Fifteen patients had pituitary adenoma; 8 had an infiltrating glioma, World Health Organization (WHO) grade 2 (6 patients had oligodendroglial components); 7 had schwannoma; and 5 had meningioma. Circumscribed astrocytomas, ganglion cell/ neurocytic tumors, and vascular tumors also occurred. The 37 patients with malignant tumors were aged 22-57 years (average 38.9 years). Twelve patients had glioblastoma multiforme (GBM); 16 had an infiltrating glioma, WHO grade 3 (7 patients had oligodendroglial components); and 4 had metastatic carcinoma or sarcoma (Ewing's). The average age for patients with WHO grade 3 infiltrating gliomas was 38 years, while GBM patients averaged 40.1 years, and patients with metastatic carcinoma averaged 51.7 years. Medulloblastoma, lymphoma and malignant teratoma also occurred. In conclusion, 1.1% of the GWVs in this database had CNS neoplasia, including various types of benign and malignant tumors. The distribution of tumor types was consistent with the age range of these patients. Over 50% of WHO grade 2 and grade 3 infiltrating gliomas had oligodendroglial components. Exposure to environmental agents such as oil smoke and pesticides was possible during the 1990-1991 Gulf War. Evaluation of the potential relationship of these agents to CNS tumor development requires correlative exposure data.

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A RARE CASE OF MESENTERIC PLEXIFORM NEUROFIBROMA ASSOCIATED WITH GOBLET CELL CARCINOID AND GANGLIONEUROMA OF THE SMALL BOWEL. Qing Li, Xiaohe Yang, Robert Ambros and Jiang Qian*. Albany Medical College, Albany, NY.

Plexiform neurofibroma (PNF) is a network-like growth of neurofibroma and usually affects large segments of a nerve imparting a "bag of worm" gross appearance to the nerve. PNF is uncommon in the gastrointestinal tract, and can be associated with ganglioneuroma. But the association of PNF with ganglioneuroma and carcinoid has not been reported in the English literature. To the best of our knowledge, this is the first report of a mesenteric PNF associated with carcinoid and ganglioneuroma of the small bowel. The patient was a 48 year-old man presented with abdominal pain, heme-positive stool, and multiple skin pigmentations. During surgery, a mass was found in the ileal mesentery involving the adjacent bowel. It consisted of a PNF, a ganglioneuroma and a carcinoid. These three tumor components were intermingled in the small bowel wall adjacent to the mesentery. More interestingly, the carcinoid was a rare variant variably termed goblet cell carcinoid, mucinous carcinoid, adenocarcinoid, or microglandular goblet cell carcinoma, which has been reported to behave more aggressively. PNF is recognized as virtually pathognomonic of neurofibromatosis type 1 (NF-1), however, the patient, family and relatives did not have any other tumors or abnormalities indicating the presence of NF-1. Despite free of symptoms for 34 months after surgery, the patient needs long-term followup due to (1) the risk of developing NF-1 and possible multiple other endocrine neoplasia, (2) the risk for malignant degeneration of PNF and (3) aggressive behavior of goblet cell carcinoid.

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PRIMARY LEPTOMENINGEAL SARCOMATOSIS (PLS). Naiel Hafez and Nitya R. Ghatak. Virginia Commonwealth University, Richmond, VA.

PLS is an exceedingly rare and highly malignant tumor predominantly occurring in children and young adults. It often masquerades as subacute or chronic meningitis clinically as well as on imaging. Diagnoses of PLS in most of the cases reported so far were established at autopsy. We now report a case of PLS in a 54-y-o man diagnosed by biopsy. The patient developed progressive headaches, nausea, vomiting and back pain. An MRI revealed numerous leptomeningeal nodular high-signal lesions with involvement of the subpial parenchyma. CSF showed protein (120mg/dL), glucose (41 mg/dL), lymphocytes (5/mm3), and no malignant cells, bacteria or fungi. With a clinical impression of granulomatous meningitis, a biopsy was performed. It showed a reticulin-rich tumor mostly composed of elongated cells with frequent mitoses and high proliferative index consistent with a sarcoma. The tumor cells were positive for vimentin, and occasionally for desmin. One week following the biopsy, the patient developed subarachnoid hemorrhage and severe cerebral edema. He died in spite of decompressive craniotomy. A complete autopsy revealed no tumor elsewhere in the body. The brain was swollen with subarachnoid hemorrhage and severe herniations. The leptomeninges were diffusely infiltrated by the tumor with extension to the brain along the Virchow-Robins spaces. Our case is very similar to the small number of PLS reported so far and emphasizes that PLS should be regarded as a distinct clinico-pathlogical entity with poor prognosis and should be included in the differential diagnosis of sub-acute meningitis.

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CASE REPORT: A TRUE MALIGNANT SCHWANNOMA OF THE EIGHTH CRANIAL NERVE. Jennifer M. Eschbacher, Sarah Estrada, Fernando Gonzalez, Gregory P. Lekovic and Stephen W. Coons*. Barrows Neurological Institute of St. Joseph's Hospital and Medical Center, Phoenix, AZ.

Although sometimes inappropriately called "malignant schwannoma," most malignant peripheral nerve sheath tumors are, in fact, malignant neurofibromas. True malignant schwannomas are exceptionally rare. Whereas eighth (vestibular) nerve schwannomas are common, there are only two reports of a malignant schwannoma at the cerebellopontine angle involving the eighth cranial nerve. We report a primary malignant schwannoma at the cerebellopontine angle showing dedifferentiation from a benign schwannoma. The patient is a 43-year old female without a history of neurofibromatosis type 1. She initially presented with years of left sided hearing difficulty, as well as the acute onset of headache, nausea and vomiting. CT demonstrated a 6.5 x 4.5 x 3.5 cm. extra-axial lesion with central necrosis within the cerebellopontine angle. A clinical diagnosis of giant eighth nerve schwannoma was made. The patient initially underwent debulking surgery and, one week later, further microsurgical resection. Microscopically, the tumor exhibited both benign and malignant cytological features. The benign-appearing areas were typical of schwannoma: moderately cellular spindle cells arranged in intersecting fasicles with compact stroma. Mitoses were not present. This area was diffusely S-100 positive. The malignant areas consisted of two patterns and included atypical epitheloid cells and markedly hypercellular fasicles of spindle cells. Numerous mitoses were identified, particularly within the spindle cell areas. Patchy S-100 positivity was also seen. Eight months later the patient remains clinically stable, with no evidence of recurrent tumor.

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CYTOKERATIN CK20 AS A MARKER FOR CROOKE'S CELLS IN PITUITARY GLANDS OF PATIENTS WITH HYPERCORTISOLISM. Jennifer M. Eschbacher and Stephen W. Coons*. Barrow Neurological Institute of St. Joseph's Hospital and Medical Center, Phoenix, AZ.

Crooke's hyaline change is a well documented response to both exogenous and endogenous glucocortocoid excess within nonneoplastic corticotrophic cells. The characteristic appearance of Crooke's cells is known to result from the accumulation of cytokeratin filaments; however, little is known about the specific keratin subtype that makes up Crooke's cells. Identification of Crooke's cells is important in distinguishing non-neoplastic gland from adenoma in Cushing's disease. In a previous study, we reported an analysis of CK7 and CK20 expression in pituitary adenomas and noted Crooke's cells in non-neoplastic gland adjacent to a corticotroph adenoma to be CK20 positive. Based on this observation, we evaluated the use of CK20 to identify Crooke's hyaline change in corticotroph adenomas and other hypercortisolic states. We analyzed CK20 expression in ten pituitary glands, including four corticotroph adenomas, one ectopic corticotroph adenoma, one pituitary carcinoma, one adrenal adenoma, one bilateral adrenal hyperplasia, and two cases of exogenous steroid treatment. Four normal pituitary glands from non-hypercortisolic patients served as controls. All ten of the hypercortisolic cases exhibited CK20 positivity in a pattern that matched the characteristic "hyaline" appearance of Crooke's cells. In contrast, no CK20 positivity was found in control glands. We conclude that CK20 is a major keratin subtype within Crooke's cells and may prove a useful and specific immunohistochemical marker in the diagnosis of pituitary corticotroph adenomas, as well as other hypercortisolic states.

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CO-EXPRESSION OF ERYTHROPOIETIN AND ERYTHROPOIETIN RECEPTOR IN VON HIPPEL-LINDAU DISEASE-ASSOCIATED TUMORS. Youn-Soo Lee[1], Alexander O. Vortmeyer[2] and Zhuengping Zhuang[2]. [1]The Catholic University of Korea, Seoul, Korea. [2]National Institute of Neurologic Disorders and Stroke, Bethesda, MD. (Sponsored by Elisabeth J Rushing*.)

Von Hippel-Lindau (VHL) disease is characterized by multiple tumors in specific target organs. The tumors at different sites share distinct morphologic and genetic characteristics but their cell of origin is unknown. In our recent studies, we have found that VHL-associated tumors are composed of developmentally arrested angiomesenchymal cells that co-express erythropoietin (Epo) and Epo receptor. In this report, we present the evidence that VHL-associated tumor including hemangioblastoma, renal cell carcinoma (RCC), and endolymphatic sac tumor (ELST) consistently co-express Epo and Epo receptor. Co-expression of Epo and Epo receptor is detected by immunohistochemistry and confirmed by RT-PCR and Western blot. In addition, co-expression of Epo and Epo receptor is also detected in many precursor lesions of hemangioblastoma and RCC such as renal cysts. While expression of Epo appears to be secondary to VHL deficiency, however, expression of Epo receptor in the hemangioblastoma, RCC and ELST may reflect a immature developmental arrest in immature mesenchymal cells in these VHL patients. Such arrest may lead to autocrine stimulation by Epo and Epo receptor in such cell, that causes cell proliferation, and eventually tumor development and progression.

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NOVEL APPROACHES TO METASTATIC ANIMAL MODELS: A PILOT STUDY FOR BRAIN METASTASIS IN MICE WITH *IN VIVO*, REAL-TIME IMAGING OF TUMOR GROWTH. Melike Mut, Joan E. Carpenter, Gerald T. Redpath, David W. Mullins, Craig L. Slingluff, Bijoy Kundu, M. Beatriz S. Lopes*, Isa M. Hussaini and Mark E. Shaffrey. University of Virginia School of Medicine, Charlotttesville, VA.

Animal models for brain tumors have provided a significant insight in understanding molecular mechanisms of disease progression and development of new treatments. Despite several models currently available for gliomas, there is no reproducible model to mimic human cancers that metastasize to brain in animals. We have developed a "brain melanoma metastasis model in mice" that may recreate the metastatic cascade of cancer. Human melanoma cell lines derived from patient's specimens with metastatic melanoma to the brain were harvested and cultured (HM1 and 86). Cells were transfected with luciferase marker gene and xenografted into 2 different groups of immunocompromised mice in this pilot study. In the first group (4 animals), tumors embedded into cellulose matrix (Gelfoam®) were injected stereotactically into brain to allow "activation" of melanoma cells in brain tissue milieu. In the second group (4 animals), tumors were injected intradermally to recreate the metastatic cascade resulting in brain metastasis. Both intradermal and intacranial injection groups were imaged for luciferase activity as reflecting tumor growth and patterns of metastasis without sacrificing the animals. Two out of 4 intradermal and 3/4 intracranial injections reveal tumor growth with detected luciferase activity. This is a pilot study that utilizes novel approaches to metastatic animal models; namely, this is the first study to mimic the metastastic cascade to the central nervous system using cancer cell lines that are "predisposed" to brain metastasis and to evaluate *in vivo* tumor growth.

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HISTIOCYTIC SARCOMA OF THE CNS: CASE REPORT OF A RARE HEMATOPOIETIC MALIGNANCY. D. G. Trembath[1], J. W. Turner[1], T. J. Cummings[1], L. R. Dibernardo[1], A. Perry[2] and C. M. Hulette[1]. [1]Department of Pathology, Duke University Medical Center, Durham, NC. [2]Washington University School of Medicine, St. Louis, MO.

Histiocytic sarcoma (HS) is a rare malignancy characterized by cells with morphologic and immunohistochemical features of mature histiocytes. We report an autopsy case of HS with extensive CNS involvement. The patient was a 60-year-old male who presented with complaints of syncope. Initial work-up revealed no neurologic abnormalities. The patient developed intractable headaches necessitating placement of a ventriculoperitoneal shunt. Laboratory testing for infectious and autoimmune etiologies were negative. The patient deteriorated, developing bifacial weakness and left hypoglossal nerve palsy. An MRI was remarkable for diffuse leptomeningeal enhancement. Brain biopsy showed reactive gliosis. The patient died 5 months after initial presentation. At autopsy, the brain demonstrated mild ventricular dilatation and meningeal congestion. Microscopically, there was an extensive subarachnoid tumor infiltrate extending into the Virchow-Robin spaces. Focal parenchymal involvement was seen in the cerebellum, with extensive invasion of the cortex and accompanying marked parenchymal gliosis. The tumor cells demonstrated moderate pleomorphism with enlarged, irregular nuclei and occasional prominent nucleoli. Rare binucleated cells were seen. The tumor cells were positive for HAM-56 and CD68, but were negative for CD20, CD3, CD30, ALK-1, S-100, CD1A, MART-1, HMB-45, cytokeratin, EMA, and GFAP. Work up for microorganisms was negative. Immunohistochemical staining for KI-67 demonstrated a proliferation index of 2%. Similar atypical histiocytes were seen in the subarachnoid space of the optic nerves. HS is a rare entity which must be differentiated from other hematological malignancies, particularly anaplastic large cell lymphoma and it should be considered in cases of unexplained meningitis with a prominent histiocytic component.

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UNUSUAL PRESENTATIONS OF HEMATOLYMPHOID PROCESSES AS PRIMARY CENTRAL NERVOUS SYSTEM (CNS) TUMORS. Stephen Hughes[1], Kenneth Fallon[2], Faruk Aydin[3], Tom O'Brien[4] and Murat Gokden[1]*. [1]University of Arkansas for Medical Sciences, Little Rock, AR. [2]Louisiana State University Medical Center, New Orleans, LA. [3]Freepath Laboratories, Tampa, FL. [4]Methodist Healthcare Central Hospital, Memphis, TN.

Involvement of CNS by neoplastic and non-neoplastic hematolymphoid processes is well-known. Lymphomas of the CNS are also well-recognized as distinct clinicopathologic entities. However, various hematolymphoid lesions can rarely present outside the characteristic clinicopathologic setting. Moreover, they may involve CNS structures only. Here, we present six rare hematolymphoid processes, with initial and unusual presentations as solitary CNS lesions, clinically and radiologically imitating primary CNS tumors. Table shows the clinical features and pathologic diagnoses.

None of the patients had any previous diagnosis of a hematolymphoid disorder, nor did further work-up after the initial neuropathologic diagnoses revealed any involvement of other sites or organs by these processes. In general, these are uncommon disorders to involve the CNS. Their presentation as isolated CNS tumors presents a challenge to the neuropathologist and underscores the importance of the awareness of these entities by the neuroradiologist, neurosurgeon, and the neuropathologist.

Case	Age/ Gender	Location of the lesion	Cilinical-radiologic diagnosis	Pathologic diagnosis
1	73/Female	Frontoparietal, parasagittal, dura-based	Meningioma	Follicular lymphoma
2	52/Female	Frontoparietal, dura-based	Meningioma	Follicular lymphoma
3	52/Female	Cavernous sinus	Schwannoma	Diffuse large B-cell lymphoma
4	73/Female	Cerebellopontine angle	Schwannoma	Anaplastic diffuse large B-cell lymphoma
5	78/Male	Frontal, dura-based	Meningioma	T-cell lymphoma
6	23/Female	Sacral bone, spinal nerves	"Sacral mass"	Rosai-Dorfman disease

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PAPILLARY TUMOR OF THE PINEAL REGION: A CASE REPORT AND REVIEW. B. I. Germin[1], R. S. Bakos[2], A. L. Petraglia[3] and J. M. Powers[1]. [1]Departments of Pathology and Laboratory Medicine and [2]Neurological Surgery; and [3]University of Rochester School of Medicine and Dentistry, Rochester, NY.

Pineal gland tumors are rare. We report a 66-year-old Caucasian male who presented with headaches. A MRI demonstrated hydrocephalus and a well-circumscribed mass in the pineal region. The resected tumor exhibited, in part, morphology consistent with a classical ependymoma with anuclear perivascular pseudorosettes. Other areas contained papillary structures with rosettes that lacked the anuclear perivascular zone. No mitotic activity or necrosis was identified. Immunostaining revealed the following: Ki-67 and p53 negative, keratin cocktail and CAM 5.2 strongly positive, glial fibrillary acidic protein (GFAP) negative, synaptophysin focally positive to equivocal to negative, vimentin positive, especially near the blood vessels, and transthyretin (prealbumin) positive. These histological and immunohistochemical characteristics confirmed the diagnosis of a papillary tumor of the pineal region, which has been recently described by Jouvet et al (2003) and Shibahara et al (2004). This tumor shares morphologic features with both papillary ependymoma and choroid plexus papilloma, and the cell of origin is believed to be a modified ependymal cell of the subcommissural organ. We present an embryological and functional review of the human subcommissural organ, a review of the literature on papillary tumors of the pineal region and finally a review of archived cases from our files.

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PAPILLARY TUMOUR OF THE PINEAL REGION IN AN ADULT WITH A PRIOR HISTORY OF EPENDYMOMA. Peter Gould[1], Katrine Grondin[2], Luc Grondin[3] and Marc Duplessis[3]. [1]CHA Hôpital de l'Enfant-Jésus; and [2]Université Laval, Quebec City, QC, Canada. [3]CHR de Trois-Rivières, Trois-Rivières, QC, Canada.

Papillary tumour of the pineal region (PTPR) is a recently recognized tumour entity thought to arise from the specialized ependyma of the subcommissural organ. Prior to the description of this entity, such tumours were most likely diagnosed as ependymomas or choroid plexus tumours. We present the case of a 31 year old male who underwent neurosurgical resection for a PTPR, 18 years after resection of an ependymoma situated in the posterior half of the third ventricle. The original pathology report from 1986 described a tumour with "pseudopapillary" structures as well as compact areas more typical of ependymoma. There was no ultrastructural evidence of surface differentiation in these areas, but ependymal type lumens were identified which contained microvilli and rare cilia. Immunohistochemistry at the time showed no staining for keratin. The patient was treated with radiotherapy and remained symptom free until 2004. The recurrent tumour showed mixed ependymal and papillary features by light microscopy, with rosettes and pseudorosettes in the compact portions of the tumour. There was strong immunohistochemical staining for keratin (CK 8/18) but sparse staining for GFAP. There was a clear demarcation between the tumour and the adjacent pineal gland, which retained its normal architecture and stained strongly for synaptophysin. Reexamination of the original tumour with current staining techniques demonstrated focal keratin in the papillary areas. The histologic and immunohistologic findings in the recurrent tumour support the diagnosis of PTPR. This case expands the spectrum of PTPR and supports its classification as an ependymal neoplasm.

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GENE-EXPRESSION PROFILING OF LUNG CARCINOMA FOLLOWING TRANSFECTION WITH TIMP-1 OVEREXPRESSION VECTOR. Amyn M. Rojiani*, Steven Brem, Marguerite Wotoczek-Obadia and Mumtaz V. Rojiani. H. Lee Moffitt Cancer Center at the University of South Florida College of Medicine, Tampa, FL.

Tissue inhibitors of matrix metalloproteinases (TIMPs) are well recognized participants in malignant progression, with a wide range of reported biological activities including inhibition of MMP activity, activation of proMMP, regulation of cell proliferation, matrix binding, angiogenesis and induction of apoptosis. Previously, we have transfected H2009, a human lung carcinoma cell line, with vector pBK-CMV hTIMP-1 resulting in HB-1, a TIMP-1 over expressing clone. When implanted into the CNS of nude mice, HB-1 cells displayed altered tumor kinetics with tumors that were larger and more aggressive in their growth patterns. Pancreatic carcinoma cells (PANC) and their TIMP-1 transfected cells (CD1) on the other hand yielded fewer and smaller tumors when similarly examined. In an attempt to identify factors that may contribute to this variable effect of TIMP-1 over expression we used a Q series human angiogenesis gene array (Superarray Bioscience Corporation). Total RNA was isolated from H2009 and HB-1 cells using the Qiagen RNeasy minikit, amplified, labeled and hybridized on to the membrane, and detected with alkaline phosphotase stepavidin/chemiluminescent detection system. Certain genes implicated in invasion and angiogenesis are seen to be upregulated. These include bFGF, hypoxia inducible factor-1, integrins alpha 5 and beta 3, TGFbeta receptor 1 and midkine. On the other hand a decrease in EGFR, MMP2, thrombospondin and TIMP2 are also noted. Decreased matrix metalloproteinase activity and increased stabilization of the matrix may further contribute to the altered tumor kinetics seen following transfection. Additional studies to further define these complex interactions are ongoing.

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ROSETTED GLIONEURONAL TUMORS OF THE SPINAL CORD. Massimo M. D'Apuzzo and Hannes Vogel*. Stanford University Medical Center, Stanford, CA.

A distinct glioneuronal tumor with rosetted neuropil islands has been recently characterized as a new mixed glioneuronal neoplasm, not defined in 2000 WHO classification of tumors of the central nervous system. Rosetted glioneuronal tumors have been predominantly described in adults, in hemispheric locations and have been associated with a possible unfavorable course (Teo *et al*., 1999). One adult example of a spinal cord rosetted glioneuronal has been previously reported. We report a spinal tumor in a 12 month old male, with expansion of the cervicothoracic spinal cord and central cyst-like rim-enhancing lesions. Pathological analysis showed a mixed glioneuronal neoplasm with prominent synaptophysin positive rosetted islands, frequently distributed in a background of bland cells with small rounded nuclei, showing mixed GFAP and synaptophysin positivity. There was a focally increased proliferation rate reaching 15 % of the neoplastic cells and glomeruloid microvascular proliferation. This report suggests that glioneuronal tumor with rosetted islands may occur in infants, thus expanding the clinical spectrum of glioneuronal neoplasia.

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EARLY PATTERN OF BRAIN ENTRY FOR VISUALIZABLE, BLOOD-BORNE MICRO-METASTASES. Lois A. Lampson[1]*, Dennis S. Meredith[1] and Cara A. Tripp[2]. [1]Harvard Medical School and [2]Brigham and Women's Hospital, Boston, MA.

As therapy improves for cancer at other sites, brain metastases are of increasing clinical importance. New models and new therapies are both needed. Our long-term goal, exploiting shared features of migratory cells, is to use lymphocytes to deliver therapy to metastatic tumor in the brain. Here we describe the early pattern of entry for visualizable, blood-borne micro-metastases in a syngeneic rat model. METHODS. MATB/lacZ, a derivative of the 13672 rat mammary carcinoma that constitutively expresses the lacZ reporter gene product, E. coli-b-galactosidase (b-gal), was injected into the left common carotid artery of syngeneic young adult female Fischer rats. After 3-7 days, rats were sacrificed by intra-cardiac perfusion with fixative (2% glutaraldehyde) and 6u cryostat sections through the brain were stained to reveal b-gal+ tumor (histochemical b-gal stain, X-gal substrate). RESULTS. In the brain proper, tumor was sporadically distributed on the injection side; it could appear as emboli within vessels or as small clusters in the brain. Tumor was also seen in vessels in the meninges and in the choroid plexus, especially in the lateral ventricle on the injection side. DISCUSSION. The pattern of tumor entry is consistent with the known behavior of human breast cancer and other tumors. It is known that lymphocytes can enter the brain by the same routes. The next step is to define the extent to which lymphocytes co-localize to sites of micro-metastases or can be made to do so. The fact that most tumor appears on the injection side provides a useful internal control.

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ACQUIRED CHIARI I-TYPE DEFORMITY SECONDARY TO PACHYMENINGEAL PROLIFERATION MIXED WITH MULTIFOCAL MENINGIOMA; REPORT OF A CASE. Mila Blaivas* and Stephen Gebarski. University of Michigan Medical School, Ann Arbor, MI.

Chiari I malformation may show minor meningeal thickening, but has not been reported associated with pachymeningeal inflammation and fibrosis or meningioma. We report a 23 year old African-American man who complained of headache, nausea and vomiting. MRI showed Chiari I-type tonsillar ectopia and brain distortion likely secondary to dramatic mainly basilar pachymeningeal lobular thickening. Reactive change, lymphoma, sarcoidosis, tuberculosis and meningiomatosis were considered in the differential diagnosis. A right pterional lobule was biopsied and revealed thick, hypercellular dura infiltrated by CD3 positive T lymphocytes, CD26 positive B lymphocytes, and polyclonal plasma cells accumulating at the periphery of the sections, around vessels, and scattered throughout the entire lesion. Numerous conspicuous rounded nodular clusters were formed by larger, epithelioid cells of meningotheliomatous and transitional meningioma. The latter stained positively with vimentin and EMA, and demonstrated a low proliferation index in the section stained for Ki67. No microorganisms or sarcoid/tuberculous granulomas were present.

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EXPRESSION OF APOPTOSIS-RELATED MOLECULES SURVIVIN, CASPASE-3, BCL-2 AND BAX IN MENINGIOMAS. Donna M. Vincenti, Wendy A. Lavezzi, Christine E. Sheehan, Jeffrey S. Ross and Jiang Qian*. Albany Medical College, Albany, NY.

Intracranial meningiomas are dura-based neoplasms. The majority of meningiomas are slow-growing tumors, but a minority can exhibit atypical features and behave more aggressively. Meningiomas are known to possess cytogenetic and molecular abnormalities, notably involving chromosome 22 and NF2 gene. Survivin and Bcl-2, anti-apoptotic proteins, and caspase-3 and Bax, promoters of apoptosis, have been implicated in tumor development and progression in a variety of neoplasms. This study was conducted to examine the role these molecules may play in meningiomas. Formalin-fixed paraffin-embedded archival tissue from 46 meningiomas (21 atypical, 25 usual) was immunohistochemically stained with antibodies against survivin, caspase-3, Bcl-2 and Bax. Positivity was scored semiquantitatively with regard to both intensity and distribution of stain. 41/46 (89%) of cases were positive for survivin, 27/46 (59%) were positive for caspase-3, 25/46 (54%) were positive for Bcl-2, and 25/46 (54%) were positive for Bax. Of 46 cases, 30 were positive for at least one pro- and one anti-apoptotic protein. 12/46 expressed all four proteins, of which 8 were atypical and 4 were usual meningiomas. This study demonstrated that the majority of meningiomas showed expression of survivin. Many expressed at least one pro- and one anti-apoptotic cell cycle regulator, and a subpopulation expressed all four markers. Although atypical and usual meningiomas could not be distinguished by expression of these markers, the data suggest that cell-cycle dysregulation may play a role in meningioma tumorigenesis.

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RHABDOID MENINGIOMA WITH DIFFUSE EXPRESSION OF GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP): A CASE REPORT. Geraldine Owor, Xiaohe Yang, Arnulf Koeppen, Eric Deshaies, Alan Boulos and Jiang Qian*. Albany Medical College, Albany, NY.

Rhabdoid meningioma is a rare, but well-established variant of meningioma and has been designated WHO grade 3 due to its aggressive clinical behavior. Its defining histopathological feature is the presence of rhabdoid cells in a discohesive growth pattern. Diagnosing such tumor may be occasionally challenging when more typical meningothelial element is not readily appreciable, or immunohistochemical profiles are confusing since this variant can produce unexpected stain patterns. Here we report one such case where unusual GFAP expression raised possibility of astrocytoma. A 57 year old woman with rheumatoid arthritis and Raynaud's syndrome presented with vomiting and severe headaches. Gadolinium-enhanced T1-weighted MRI demonstrated a 5.5 x 4.5 x 7cm left calvarial mass arising from the posterior falx with one component appearing more aggressive. Histopathology revealed a neoplasm with a major component of rhabdoid cells and minor components of clear cell and papillary differentiation. Meningothelial nature was only vaguely discernible in focal areas of solid diffuse sheets. Rhabdoid cells formed loosely-cohesive clusters, cords, or individual files and diffusely infiltrated dura. Superficial brain invasion was present. Immunohistochemically, GFAP strongly and diffusely stained rhabdoid cells, while epithelial membrane antigen was essentially negative in rhabdoid area and only focally positive in non-rhabdoid areas. Ultrastructural study confirmed meningothelial feature in the non-rhabdoid area demonstrating intercellular junctions and interdigitating cellular processes. This case illustrated that by combining light microscopic features and ultrastructural findings in addition to clinical information such as tumor location, correct diagnosis of rhabdoid meningioma can be made despite unusual immunohistochemical profiles.

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UNUSUAL SPINDLE CELL NEOPLASM OF SPINAL CORD WITH GIANT COLLAGEN ROSETTES. Karen M. Weidenheim*, Juan Carlos. Alzate, Josefina Llena, Jacqueline Bello and Rick Abbott. Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY.

Schwannomas occurring on the spinal nerve roots are common, benign neoplasms with a characteristic histological, immunohistochemical and ultrastructural features that readily distinguish them from other spindle-cell tumors that may arise in this location, including rare fibroblastic neoplasms of the central neuraxis. Herein, we report a 17-year-old male with progressive scoliosis of 11 years duration, and lower extremity weakness for 7 years. At surgery, an intra-axial tumor extending from T7 to T10, unattached to dura and largely exophytic, was partially resected. Light microscopy revealed a moderately cellular proliferation of elongated to stellate spindle cells in a loose collagenous matrix, with uniform vesicular nuclei. A biphasic pattern, palisaded nuclei or Verocay bodies were not present. Giant collagen rosettes unassociated with peripheral hypercellularity or with inflammatory activity were present. There was no necrosis, mitotic activity or staining for MIB-1. Reticulin and collagen were present in the tumor matrix. Immunohistochemistry revealed that the tumor cells were strongly positive for CD34 and moderately positive for S100. The tumor cells were negative for bcl2, desmin, smooth muscle actin and epithelial membrane antigen. Electron microscopy revealed that some but not all of the spindle cells possessed external lamina, and contained rough endoplasmic reticulum and occasional mitochondria. Pinocytosis and intercellular junctions were not present. Gliotic, disorganized central nervous system tissue nests, leptomeninges, peripheral nerve fascicles, and adipose tissue were intimately associated with the spindle cells. This unusual neoplasm has both fibroblastic and Schwannian features as well as giant collagen rosettes, and has so far exhibited benign behavior.

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AGGRUS EXPRESSION IN PRIMARY INTRACRANIAL GERMINOMA. Eyas M. Hattab*, Beamon Agarwal, Romil Saxena and Sunil Badve. Indiana University Medical Center, Indianapolis, IN.

Background: Aggrus is a newly identified 44 kDa sialoglycoprotein overexpressed on the surface of tumor cells. Its physiological role has yet to be determined and its function in tumors is not well characterized. However, it was recently proposed that Aggrus functions as an adhesion molecule that promotes platelet aggregation in pathological conditions. Aggrus has been shown to be upregulated in colorectal carcinomas and a recent report demonstrated its expression in testicular seminomas. In this study, we investigate whether a similar pattern of expression is observed in primary intracranial germinomas. **Methods:** The archival pathology files of the Indiana University Medical Center were searched, over a 20-year period, for primary germinomas of the central nervous system. The search yielded 20 cases in which paraffin blocks with sufficient material were available. All cases were reviewed and sections were obtained and immunostained for Aggrus (Angiobio, Del Mar, CA, dilution 1:100). Stained sections were graded in a semi-quantitative/qualitative fashion as follows: negative (<10% of tumor cells staining), 1+ (mild intensity), 2+ (moderate intensity), 3+ (intense staining) **Results:** Aggrus immunohistochemical staining was detected in 19 of 20 cases (95%), with the large majority (17/19, ~90%) demonstrating intense (3+) and often diffuse membranous and cytoplasmic staining. **Conclusion:** Aggrus is a highly expressed immunohistochemical marker in primary intracranial germinomas. Aggrus may play a role in tumorigenesis of primary intracranial germinomas and be useful for their diagnosis.

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A FATAL CASE OF POSTPARTUM CEREBRAL ANGIOPATHY WITH REVIEW OF THE LITERATURE. Timothy L. Williams, Timothy G. Lukovits, Brent T. Harris and C. Harker. Rhodes*. Dartmouth-Hitchcock Medical Center, Lebanon, NH.

Introduction: Postpartum cerebral angiopathy (PCA) is a rare and pathophysiologically ill-characterized cerebral vasoconstriction syndrome occurring within 30 days of a usually uncomplicated pregnancy and delivery. Its onset has been associated with the use of vasoactive medications, particularly ergot alkaloids. Other cases have occurred in the absence of these medications (spontaneous cases), prompting conjecture into possible overlap between PCA and other conditions known to cause cerebral vasoconstriction, including primary angiitis of the central nervous system and postpartum eclampsia. The vast majority of cases follow a relatively benign course; however, a fatal case has been reported. Histopathologic findings in PCA, so far limited to the fatal case and two more recent biopsies, have been nonspecific. **Objective:** Here we present a second fatal case of PCA, including pre- and post-mortem histopathologic analysis. We also include a review of all PCA cases reported in the English literature. **Methods:** Criteria for the clinical diagnosis of PCA are proposed and used to select case reports from the medical literature. Data pertaining to patient characteristics, clinical symptomatology, cerebral imaging findings, and clinical outcomes are compared between cases associated with the postpartum use of vasoactive medications and spontaneous cases. **Conclusions:** Histopathologic findings in PCA are nonspecific and secondary to ischemic brain injury. Functional vasoconstriction is the most likely primary pathophysiologic process in PCA. The etiology in cases associated with medications may be due to idiosyncratic reactions to these agents. Significant overlap in symtomatology and clinical findings exists between spontaneous cases and late postpartum eclampsia.

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NEURONAL EXPRESSION OF αB CRYSTALLIN (αBC) IN THE PSEUDOHYPERTROPHY OF THE INFERIOR OLIVES. Katsuhiko Ogawa[1], Kenji Uehara, Masayuki Minami[1], Yoshio Suzuki[2] and Tomohiko Mizutani[1]*. [1]Nihon University School of Medicine, Tokyo, Japan. [2]Asahi General Hospital, Chiba, Japan.

αBC is expressed in ballooned neurons, but not in normal neurons. Since we found αBC immunoreactivity in some neurons of the degeneration of the inferior olives, we investigated αBC neuronal expression in the inferior olives of 45 brains with various neurological diseases. We studied their inferior olives by HE, Klüver-Barrera, Bodian and Gallyas-Braak stainings, and immunohistochemical staining using antibodies to αBC, synaptophysin and GFAP as well as SMI 31. We found occasional to frequent αBC neuronal expression in all 8 brains with pseudohypertrophy caused by cerebrovascular diseases, 2 out of 4 with multiple system atrophy, 1 out of 3 with progressive supranuclear palsy or corticobasal degeneration, 1 out of 11 with motor neuron disease, all 2 with paraneoplastic cerebellar degeneration, 2 out of 3 with tuberculous meningitis, and 1 with chronic encephalitis of the brainstem and cerebellum, but not in any of 11 Parkinson disease and dementia with Lewy bodies, and 3 with Machado-Joseph disease. We demonstrated for the first time that neuronal expression of αBC occurred frequently in the pseudohypertrophy of the inferior olives. Since this neuronal expression was, if any, infrequent in the inferior olives affected by the neurodegenerative diseases as above, we speculate that αBC expression may occur in abnormal neurons which tend to swell, not in atrophic neurons. αBC is a stress protein and plays a protective role as molecular chaperone. This may have caused αBC neuronal expression in the pseudohypertrophy of the inferior olives.

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BRAIN TRANSPLANTATION OF IMMORTALIZED HUMAN BONE MARROW MESENCHYMAL STEM CELLS IN RATS WITH INTRACEREBRAL HEMORRHAGE STROKE. S. U. Kim[1,2], W. K. Kim[1], A. Nagai[2], H. J. Lee[1], S. H. Hong[1], K. S. Kim[1], Y. K. Jang[1], I. H. Park[1] and S. W. Jeong[3]. [1]Brain Research Institute, Ajou University School of Medicine, Suwon, Korea. [2]Department of Neurology, University of British Columbia, Vancouver, Canada. [3]Department of Neurology, Ilsan Paik Hospital, Goyang, Korea.

Bone marrow mesenchymal stem cells (MSCs) have multilineage differentiation capacity to become various cell types including neurons. Stable clones of immortalized human MSCs were generated by transfection with a retroviral vector encoding *v-myc*. B10, one of the lines, carries normal human karyotype of 46,XX and expresses human MSC cell type markers CD13, CD29, CD44, CD49b, CD90 and CD166. When grown in differentiation media, more than 60-80% of B10 MSCs differntiate into osteocytes, chondrocytes, adipocytes or neurons. Following treatment with bFGF, More than 30% of B10 MSCs differentiated into cells with neuronal lineage cells (β-tubulin III, NF-L, NF-M, neuN). A small number (<1%) of B10 cells expressed GFAP or CNPase. B10 MSCs also expressed genes involved in neural differentiation including Oct4, Otx2, Mash1, ABCG and nestin as determined by RT-PCR. Following intraventricular transplantation in neonatal mice, B10 cells migrated extensively and differentiated into neurons in hippocampus, neocortex and striatum. Two to 12 weeks following transplantion in striata of intracerebral hemorrhage (ICH) stroke models in adult rats and mice, B10 cells induced a marked functional improvement. B10 cells were found to migrate into lesion sites, and differentiate into nerurons and astrocytes. The replacement of lost neurons and production of BDNF and other neurotrophic factors by grafted human MSCs are underlying attributes of functional restoration in rat stroke models. Immortalized human MSCs should serve as a powerful tool for research into development of stem cell based therapy for human neurological disorders.

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BRAINNET EUROPE–A CONSORTIUM OF EUROPEAN BRAIN BANKS SERVING THE NEUROSCIENCE RESEARCH COMMUNITY. J. E. Bell[1], I. Alafuzoff[3], S. Al. Saraj[4], N. Bogdanovic[5], W. Brück[6], H. Budka[7], P. Falkai[8], I. Ferrer[9], G. Giaccone[10], J. J. Hauw[11], N. Kopp[12], G. Kovacs[13], M. Palkovitz[14], P. Parchi[15], E. Patsouris[16], R. Ravid[17], R. Reynolds[18], P. Riederer[19] and H. Kretzschmar[2]. [1]Division of Pathology, University of Edinburgh, UK. [2]Institute of Neuropathology, University of Munich, Germany. [3]Department of Pathology, University of Kuopio, Finland. [4]Department of Neuropathology, King's College London, UK. [5]Geriatric Department, Karolinksa Institute, Stockholm, Sweden. [6]Bereich Humanmedizin Georg-August, University of Gottingen, Germany, [7]Institute of Neurology, University of Vienna, Austria. [8]Department of Psychiatry & Psychotherapy, University of Saarland, Germany. [9]Institute of Neuropathology, University of Barcelona, Spain. [10]Department of Neurology & Neuropathology, University of Milan. [11]Institut National de la Santé et de la Recherche Medicale, Paris, France. [12]Department Neuropathology, University of Lyon, France. [13]National Institute of Psychiatry & Neurology, University of Budapest, Hungary. [14]Joint Laboratory of the Hungarian Academy of Science and Semmelweis, University of Budapest, Hungary. [15]Department of Science & Neurology, University of Bologna, Italy. [16]Department of Neuropathology, University of Athens, Greece. [17]Netherlands Brain Bank, Netherlands Institute for Brain Research of the Royal Netherlands Academy of Sciences, Amsterdam, Netherlands. [18]Multiple Sclerosis Brain Bank, Imperial College of Science Technology & Medicine, London, UK. [19]Department of Psychiatry, University of Wuerzburg, Germany.

Since 2001, brain banks in 19 different European centres have been working together to establish a Consortium which has several aims. These include optimisation of methodology applicable to brain banking, not only in tissue preparation but also in experimental approaches such as proteomics and tissue microarray. The Consortium is working to clarify the different legal and ethical requirements which govern brain banking in the countries involved, in order to optimise international alignment of brain banks. The Consortium will offer research samples for use through a web-based port (http://www.brainnet-europe.org/) and will produce best practice guidelines for all the techniques associated with brain banking. A training programme will be established in order to ensure the future of high quality brain banking since this is recognised as an essential resource infrastructure for the neuroscience community. The Consortium is now fully operational, funded by the European Community Framework 6 Programme. An international Management Board is in place and this includes US experts in brain banking.

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A CASE REPORT OF RHEUMATOID LEPTOMENINGITIS AND PACHYMENINGITIS. Di Tian[1], Richard C. Chou[2], John W. Henson[3], Anthony M. Reginato[2] and E. Tessa. Hedley-Whyte[1]*. [1]C.S. Kubik Laboratory for Neuropathology, Department of Pathology; [2]Division of Rheumatology, Allergy, and Clinical Immunology, Department of Medicine; [3]Stephen E. and Catherine Pappas Center for Neuro-oncology, Department of Radiology; Massachusetts General Hospital, Harvard Medical School, Boston, MA.

Rheumatoid meningitis/pachymeningitis is a rare extra-articular manifestation of rheumatoid arthritis (RA) and usually fatal. Here we report the clinical and neuropathological features of a patient with this entity. She was 58-year-old with a history of fibromyalgia, osteoarthritis, and chronic migraine headache. She initially had episodic weakness and numbness in her left leg and left upper extremity one month before her admission to Massachusetts General Hospital (MGH). Shortly afterwards, she developed fever, worsening polyarthritis involving both hands, wrists, knees, and ankles, severe headache, recurrent falls, intermittent dysphagia, slurred speech, and right-sided visual change. She was admitted to MGH for evaluation and treatment. A brain MRI showed loss of gray/white matter differentiation and enhancement of the leptomeninges of the right frontal-parietal lobe. These abnormalities raised the possibility of either viral or carcinomatous leptomeningitis. Lumbar puncture showed mildly increased white blood cell count with 78% lymphocytes, 22% monocytes and no evidence of malignancy. Her serological studies and radiographic findings were consistent with seropositive RA. A brain biopsy revealed thickening of dura and leptomeninges with dense infiltration by mature B- and T-lymphocytes, plasma cells, epithelioid histiocytes, and several multinucleated giant cells. An area of necrosis was also present. No infectious agents, malignant cells, or vasculitis were recognized. The overall histological findings were consistent with rheumatoid leptomeningitis/pachymeningitis. She was successfully treated with cyclophosphamide and oral prednisone with complete resolution of her neurological symptoms and neuroimaging abnormalities. A review of clinical and neuropathological features of rheumatoid meningitis / pachymeningitis is discussed.

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OLIG2 FUNCTION IS REQUIRED FOR NG2 CELL DEVELOPMENT. K. L. Ligon[1]*, T. Sun[3], H. A. Arnett[2], M. Kitada[2], J. A. Alberta[2], C. D. Stiles[2] and D. H. Rowitch[2]. [1]Brigham and Women's Hospital; and [2]Dana-Farber Cancer Institute, Children's Hospital Boston, Harvard Medical School; and [3]Beth Israel Deaconess Hospital, Boston, MA.

NG2 proteoglycan expressing cells are an abundant cell type within the brain but their developmental lineage and functions are poorly understood. Although certain populations of NG2 cells exhibit similarities to oligodendroglial precursor cells, recent studies have raised questions about whether the unique properties of NG2 cells might warrant their designation as a "fourth" novel cell type of the CNS. The transcription factors Olig1 and Olig2 are required for specification and development of all oligodendroglial cell types throughout the nervous system and we therefore used Olig2 knock-out mice to test the genetic requirement of these genes for NG2 cell development. Immunohistochemical co-localization of Olig2 and NG2 in normal mouse brain and spinal cord showed that NG2+ cells co-express Olig2 at embryonic day 18.5. In vitro differentiation of rat cortical neuroepithelial precursor cells in the presence of FGF also demonstrated that greater than 90% of NG2 cells co-express Olig2. Analysis of the brain and spinal cord of Olig2 null mice at E18.5 showed no detectable NG2 expression in cells with typical morphology. NG2 cell development was rescued upon mating of Olig2 null mice with BAC transgenic lines expressing human Olig1 and Olig2. These data suggest that prenatal NG2 cells require Olig2 function for their development and are derived from the Olig2 defined oligodendroglial lineage.

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A SONIC HEDGEHOG GLYCINE RESIDUE MUTATED IN HUMAN HOLOPROSENCEPHALY IS REQUIRED FOR LIGAND MULTIMERIZATION AND LONG-RANGE SIGNALING. Harvard Medical School Boston, MA. (*Sponsored by Keith L. Ligon.)

Sonic Hedgehog (SHH) is an extracellular ligand that functions as a morphogen and mitogen in diverse tissues. Mutations that reduce SHH activity cause holoprosencephaly, whereas mutations that activate SHH pathway signaling can lead to development of medulloblastoma. During neural tube and limb development, a gradient of SHH protein specifies the patterning of responsive tissues. The specific biologic responses elicited depend on the local SHH concentration perceived by recipient cells based on their positions within the gradient. Furthermore, the ability of the protein to aggregate into soluble multimeric forms may be important for SHH spreading through tissues in order to form the gradient. Here we show that a SHH mutation that causes human holoprosencephaly (G31R) results in decreased ligand multimerization in vitro. We used partially-denaturing and non-denaturing polyacrylamide gel electrophoresis to analyze multimer formation of wild-type and mutant SHH overexpressed in HEK cells. The mutant protein is normally processed and lipid-modified, however the relative amount of multimeric forms is reduced compared to the wild-type protein. We also show that the G-to-R mutation results in decreased long-range SHH activity in vivo. We generated knock-in mice harboring the equivalent mouse SHH mutation (mouse G32R) to examine in vivo effects. G32R homozygous mutants are late embryonic lethal and show failure of ventral neural tube induction, holoprosencephaly, and abnormal specification of digits 2 and 3. Our data support a model in which normal SHH multimer formation is necessary for appropriate long-range signaling, and suggest a pathophysiologic mechanism for one of the known human holoprosencephaly mutations.

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GENOTYPICALLY DEFINED LISSENCEPHALIES SHOW DISTINCT PATHOLOGIES. Mark S. Forman[1], Marian Squier[2], William B. Dobyns[3] and Jeffrey A. Golden*[4]. [1]University of Pennsylvania School of Medicine, Philadelphia, PA. [2]Radcliffe Infirmary, Oxford, UK. [3]University of Chicago, Chicago, IL. [4]Children's Hospital of Pennsylvania and the University of Pennsylvania School of Medicine, Philadelphia, PA.

Lissencephaly is traditionally divided into two distinct pathologic forms; classical (type I) and cobblestone (type II). To date, mutations in four genes, *LIS1*, *XLIS, RELN,* and *ARX,* have been associated with distinct type I lissencephaly syndromes. Each of these genes has been shown to play a role in normal cell migration, consistent with the presumed pathogenesis of type I lissencephaly. Based on these data, we hypothesized that all forms of radiographically defined type I lissencephaly independent of genotype, would be pathologically similar. To test this hypothesis we examined the brains from 15 lissencephalic patients; 6 with *LIS1* deletions, 2 with *XLIS* mutations, and 2 with an *ARX* mutation, 5 patients had no defined genetic defect. In all cases, the cortex was thickened, however, the topographic distribution of the cortical pathology varied, ranging from frontal to occipital-biased pathology to diffuse involvement of the neocortex. While brains with *LIS1* deletions exhibited the classic 4-layer architecture, patients with *XLIS* and *ARX* mutations each had unique cytoarchitectural findings distinct from *LIS1*. Two of the 5 patients with no known genetic defect showed a fourth type of histopathology. Interestingly, the two brains with the fourth type of lissencephaly showed profound brainstem and cerebellar abnormalities. In summary, we identified at least 4 distinct histopathologic subtypes of lissencephaly that correlate with the underlying genetic defect.

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P53 AND PUMA REGULATION OF NEURAL PRECURSOR CELL DEATH. K. A. Roth*, Y. Geng and R. S. Akhtar. The University of Alabama at Birmingham, Birmingham, AL.

Neural precursor cells (NPCs) are abundant in the developing brain and reside in select locations in the adult brain. Regulation of NPC death is critically important for normal brain morphogenesis and altered NPC death in the adult brain may be involved in neurodegenerative and psychiatric disease. To begin to define the molecular pathways regulating NPC death, we have examined the response of NPCs derived from wild-type and gene-disrupted mice to genotoxic insults. NPCs from either the embryonic telencephalon or the neonatal cerebellum were expanded *in vitro* in the presence of fibroblast growth factor-2 and exposed to cytosine arabinoside (AraC) or other chemotherapeutic agents such as bleomycin, etoposide, and camptothecin and NPC caspase-3 activation and death were assessed using a variety of techniques. Both telencephalic and cerebellar NPCs showed markedly increased caspase-3 activation and apoptosis six to 24 hours after exposure to genotoxic agents. In comparison with wild-type and heterozygous littermate-derived NPC controls, p53-, Puma-, Bax/Bak-, Apaf-1-, and caspase-9-deficient NPCs showed significantly decreased caspase-3 activation and death when exposed to genotoxic agents. Treatment of wild-type mice with AraC *in vivo* resulted in extensive caspase-3 activation and NPC apoptosis. In contrast, mice deficient in Puma, or other downstream apoptosis mediators, showed minimal caspase-3 activation and NPC apoptosis but exhibited a dramatic increase in nuclear p53 immunolocalization following AraC treatment. These results suggest that Puma lies downstream of p53 in this death pathway and may be the critical p53-regulated gene controlling activation of the intrinsic apoptotic death pathway in genotoxically-injured NPCs.

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UNIPOLAR BRUSH CELLS OF THE CEREBELLUM ORIGINATE FROM THE RHOMBIC LIP/GERMINAL TRIGONE AND MIGRATE THROUGH CEREBELLAR WHITE MATTER. Robert F. Hevner*, Chris Englund, Andrew J. Fink, Ray AM. Daza and Diane Pham. University of Washington, Seattle, WA.

Unipolar brush cells (UBCs) are a type of glutamatergic interneuron located in the internal granule cell layer of the cerebellum. They are most abundant in lobules IX and X, i.e., the vestibulocerebellum. Our goal in the present study was to identify the embryonic source and migratory pathways of UBCs. Their origins and migrations were revealed by high–level expression of Tbr2, a T–domain transcription factor expressed in UBCs and their progenitors. In the late embryonic cerebellum, high–level Tbr2 immunoreactivity labeled mitotically active cells in the rhombic lip. As development progressed, Tbr2–immunoreactive cells passed through the cerebellar white matter, avoiding the deep nuclei, and came to rest in the internal granule layer. The migration was confirmed in vitro using an embryonic cerebellar slice explant system, in which the rhombic lip was replaced with *tau–GFP* transgenic rhombic lip. After 2-4 days in vitro, numerous *tau–GFP*+ cells migrated from the rhombic lip into the cerebellum. The migratory cells expressed UBC markers, including Tbr2 and calretinin. In ongoing experiments, the effects of rhombic lip ablation are being studied. Our results suggest that UBCs are produced by the rhombic lip during the same time interval as external granule cells, another rhombic lip derivative.

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THE SCOTTISH PERINATAL NEUROPATHOLOGY STUDY: APOE POLYMORPHISM, PERINATAL MORTALITY AND BRAIN INJURY. J. E. Bell1*, J.-C Becher†, J. W. Keeling* and N. McIntosh†. Division of Pathology and †Division of Child Life & Health, University of Edinburgh, UK.

A population-based study of Scottish perinatal deaths (24 weeks gestation to 1 week postnatal, n = 745) was conducted over 2 years, funded by the Scottish Home & Health Department. Full neuropathological studies were undertaken with research consent in 70 liveborn and 191 stillborn babies. Detailed clinical data were available, including history of birth asphyxia. The ApoE status was determined in each infant. 57% (n = 26) of liveborn babies with a history of asphyxia, versus 8% of the non-asphxiated group, showed prenatal brain damage (gliosis and macrophage accumulation) ($p < 0.005$). 66% of stillborn babies (n = 191) showed evidence of hypoxic damage linked to pregnancy-induced hypertension ($p = 0.08$) and low placental weight ($p = 0.002$). βamyloid precursor protein (βAPP) immunopositivity proved a useful marker of white matter damage, present in axons in 27% of neonatal deaths and correlating significantly with birth asphyxia. Hypoxia/ischaemia was considered the likely cause of βAPP positivity in these infants although birth-related trauma cannot be ruled out. There was no apparent link between ApoE status and presence/absence of brain damage or of brain haemorrhage in this cohort. However, overall, ApoE2 was over-represented among perinatal deaths (13% v 8%, $p = 0.012$). ApoE4 was over-represented in both neonatal deaths and in healthy liveborns (both 19% v 15% in healthy adults $p = 0.026$). The factors which contribute to vulnerability for hypoxic/ischaemic encephalopathy in the developing brain remain elusive and do not include ApoE status. However ApoE allele distribution varies between perinatal deaths, healthy liveborns and healthy adults.

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DANDY-WALKER MALFORMATION: CORRELATION OF DIAGNOSIS ON PRENATAL ULTRASOUND AND ON PATHOLOGY REVIEW AT AUTOPSY. Joanna J. Phillips[1], Barry Mahony[2], Joseph R. Siebert[3], Tasneem Lalani[4], Corinne Fligner[4] and Raj Kapur[3]. [1]University of California, San Francisco, San Francisco, CA. [2]Swedish Hospital; [3]Children's Hospital and Regional Medical Center; and [4]University of Washington, Seattle, WA. (Sponsored by Andrew Bollen[1]*.)

Children with Dandy-Walker malformation or Dandy-Walker variant (DWM/V) demonstrate a spectrum of outcomes and the diagnosis on prenatal ultrasound can be difficult. Forty-four cases of fetal DWM/V diagnosed by prenatal ultrasound that subsequently underwent autopsy examination between 1995 and 2003 are reported. The ultrasound and pathology data were reviewed in a blinded manner to (1) assess the concordance between the diagnosis at prenatal ultrasound and at autopsy and (2) to identify specific features associated with concordance. The original concordance between the ultrasound and the pathologic diagnosis of DWM/V was 18/44 cases (41%). A total of 17/44 cases (39%) had no evidence of DWM/V on pathologic evaluation and 9/44 cases (20%) were indeterminate. The method of termination of pregnancy was an important factor as 44% (4/9) of the cases with termination by KCL injection had indeterminate pathology. Of the 15 cases with adequate ultrasound and pathology data for review 9/15 (60%) had a concordant diagnosis of DWM/V. Features identified on ultrasound, associated with a concordant diagnosis of DWM/V, included increased depth of the cisterna magna and absence of the superior vermis. On pathology review, a concordant ultrasound and pathologic diagnosis of DWM/V was associated with the presence of a posterior fossa cyst and the absence of the superior vermis. The findings confirm that the correlation between the prenatal ultrasound diagnosis and the pathologic diagnosis of DWM/V is relatively low. Furthermore, specific features are identified on ultrasound and pathology that are associated with a concordant diagnoses of DWM/V.

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THREE DISTINCT POPULATIONS OF GIANT CELLS IN TUBEROUS SCLEROSIS AND FOCAL CORTICAL DYSPLASIA. DouglasCMillerNew York University Medical Center New York, NY.

Cerebral lesions of Focal Cortical Dysplasia and Tuberous Sclerosis Complex show numerous balloon cells (giant cells with variably astrocytic or neuronal appearance). To assess the character of these cells, 28 cases of either FCD or TSC were evaluated immunohistochemically with glial markers (vimentin and Glial Fibrillary Acidic Protein), and neuronal markers (Neu-N, synaptophysin, and Neurofilament Protein/RMDO20), including double stains of serial sections with vimentin and Neu-N; RMDO20 and Neu-N; and synaptophysin and Neu-N. As expected normal neocortical neurons were immunopositive for RMDO20 and Neu-N within a background of granular synaptophysin staining, and astrocytes with delicate fibrillary processes were immunopositive for both vimentin and GFAP. Balloon cells were almost never GFAP immunopositive, but were variably immunopositive for Neu-N, RMDO20, vimentin and synaptophysin. However, double stains of serial sections reveal three distinct populations of such cells. One population of balloon cells is RMDO20+/Neu-N+; another is RMDO20+/Neu-N-. These latter cells are vimentin positive. A third distinct population of balloon cells is RMDO20-/Neu-N- but immunopositive for vimentin. Balloon cells immunopositive for vimentin are all immunonegative for Neu-N. Neu-N immunopositive balloon cells have strong synaptophysin perikaryal staining, not seen around any Neu-N negative cells. These findings suggest the presence of diverse subpopulations of balloon cells, some with a predominance of neuronal differentiation, some mixed glial-neuronal, and a population that may be entirely glial in nature.

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HIPPOCAMPAL MICROVASCULATURE ATTRITION AND COGNITIVE DECLINE IN ALZHEIMERS DISEASE. T. L. Bailey[1], C. B. Rivara[1], D. P. Perl[1], V. Haroutunian[1], C. Bouras[2], P. Giannakopoulos[2] and P. R. Hof[1]*. [1]Mount Sinai School of Medicine, New York, NY. [2]University of Geneva School of Medicine, Switzerland.

Background: Localized alterations in brain microvasculature may account for decreased perfusion and neuronal loss in Alzheimers disease (AD). However, measurements of these microvascular changes have yet to be quantified. **Purpose:** To estimate microvascular length in hippocampal fields CA1, CA2-3, dentate gyrus, subiculum, and entorhinal cortex of demented AD and non-demented matched controls and to assess the relationships between neuropathological severity and cognitive status. **Methods:** Analysis was completed on 32 cases 59 to 102 years old. Cognitive status, measured using CDR (Clinical Dementia Rating) and MMSE score, was obtained at least 6 months prior to autopsy. Braak staging, a measure of neuropathologic severity, was done postmortem. Immunohistochemistry with collagen IV was used to visualize microvessels and Nissl staining allowed delineation of the regions of interest. Stereologic techniques were used to estimate total microvessel length. **Results:** A decrease in the total length of the vasculature in the CA1 field with increasing CDR ($p < 0.01$) and MMSE scores($p < 0.01$) was observed. Decrease in the total length of the microvasculature occurred in the CA1 field with increasing Braak stage ($p < 0.01$). Total length was not related to age in CA1. No significant changes were seen in respect to total length and CDR, MMSE, Braak score and age in the other hippocampal fields. **Conclusions:** These data provide evidence for a relationship between the length of microvessels in the CA1 field and changes in cognition in AD patients. Cognition may be influenced by changes in the microvasculature in CA1 as AD progresses. Supported by NIH grants AG02219 and AG05138, the National Medical Fellowship, and the Doris Duke Clinical Research Fellowship.

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CEREBROVASCULAR SMOOTH MUSCLE ACTIN IS INCREASED IN POSSIBLE ALZHEIMER DISEASE BRAIN TISSUE: A CONTROLLED AUTOPSY STUDY. Christine M. Hulette, John F. Ervin, Yvette Edmonds, Nicolas Stewart, K. Welsh-Bohmer, Carl F. Pieper, Mari H. Szymanski and Donald E. Schmechel. Duke University Medical Center, Durham, NC.

Studies of brain microvasculature have demonstrated that vascular smooth muscle actin is reduced in Alzheimer Disease (AD) brain compared to cognitively and neuropathologically normal controls. (J Neuropathol Exp Neurol.2004;63:735-41). Findings in additional control groups, Possible AD and Frontotemporal Dementia (FTD), are reported. Groups were matched for sex and age. All FTD and Possible AD cases were ApoE 3,3. Smooth Muscle cell Actin (SMA) immunohistochemistry and image analysis was used. Vascular SMA in the arachnoid, cortex and white matter of the FTD group was similar to the Normal Control group and therefore different from AD. Although FTD subjects had dementia and neurofibrillary tangle pathology similar to AD, they had normal vascular SMA. The "Possible AD" group were cognitively normal at death, but exhibited frequent plaques at autopsy. Surprisingly, Possible AD vessels had more SMA immunoreactivity than the normal Controls and much more SMA than AD. These individuals exhibited a "super normal" pattern of vascular smooth muscle actin immunoreactivity relative to controls who were cognitively normal at death but had no plaques at autopsy. The presence of frequent plaques, normal cognition and super abundant vascular smooth muscle actin would suggest that increased vascular smooth muscle actin may protect against the development of dementia. Loss of vascular smooth muscle actin may be an early step in the pathogenesis of Alzheimer's dementia. Supported by PHS P50 AG05128.

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HYPERHOMOCYSTEINEMIC TRIPLE TRANSGENIC ALZHEIMER'S MOUSE MODEL SHOWS INCREASED BRAIN AMYLOID BETA PEPTIDE LEVELS AND INCREASED BETA-SECRETASE ACTIVITY. Javier Pacheco-Quinto[1,3], Elena Rodriguez de Turco[1], Lorenzo Refolo[2], Nicolas G. Bazan[1], Altovise Howard[1], Felix Cruz-Sanchez[3], Steven De Rosa[4], Suzana Petanceska[4] and Miguel A. Pappolla.[1]. [1]LSU Health Sciences Center, New Orleans, LA. [2]NINDS, Bethesda, MD. [3]University of Catalunya, Barcelona, Spain. [4]Nathan Kline Institute, Orangeburg, NY.

Recent epidemiological and clinical data suggest that elevated homocysteine levels may increase the risk of developing Alzheimer's disease (AD), but the underlying mechanisms are unknown. We tested the hypothesis that high homocysteine levels affect amyloid beta-peptide (AB) levels in the brain and could therefore accelerate AD neuropathology. For this purpose, we developed a hyperhomocysteinemic triple transgenic APP/PS1/CBS mice containing a heterozygous dominant mutation in cystathionine-beta-synthase (CBS). Triple transgenic mice showed consistent and highly significant elevations of serum homocysteine levels. Compared to the double transgenic APP/PS1 model of amyloidosis, female (but not male) APP/PS1/CBS mice showed significant elevations of AB 42 and AB 40 levels and increased beta-secretase activity. Correlations between homocysteine and AB levels were statistically significant. By unveiling a link between homocysteine and AD neuropathology, these findings advance our understanding regarding the mechanisms involved in hyperhomocysteinemia as a risk factor for AD.

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NEUROTOXICITY FROM MURINE GLIAL INNATE IMMUNE RESPONSE IS GREATEST FOLLOWING TARGETED REPLACEMENT WITH HUMAN EPSILON-4 ALLELE OF THE APOLIPOPROTEIN E GENE AND IS MEDIATED BY MICROLGIAL P38MAPK ACTIVITY. Thomas J. Montine* and Izumi Maezawa. University of Washington, Seattle, WA.

Inheritance of *APOE* alleles is associated with varying clinical outcomes in several neurodegenerative diseases that are associated with innate immune response in brain. We tested the hypothesis that apoE isoforms may act by modulating glial innate immune response and thereby alter bystander damage to neurons. We first used dissociated cultures of wild type (wt) murine neurons and glia derived from mice with targeted replacement (TR) of the ε2, ε3, or ε4 *APOE* allele. Our results showed that glial innate immune response produced bystander damage to wt neurons that was greatest with TR APOE4 glia, intermediate from TR APOE3 glia, and least from TR APOE2 glia, and preceded detectable nitric oxide secretion. TR APOE2 astrocytes displayed greater basal and proportionately stimulated NF-κB activity compared to TR APOE4 astrocytes. In contrast, TR APOE4 microglia had the greatest p38MAPK-dependent cytokine secretion. In hippocampal slice cultures, glial innate immune activation was greatest in TR APOE4 cultures and produced post-synaptic neuronal damage in TR APOE4, but not TR APOE2, cultures that was p38MAPK-dependent. These findings suggest a new mechanism by which inheritance of different *APOE* alleles may influence the outcome of neurodegenerative diseases associated with glial innate immune response.

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PASSIVE IMMUNIZATION WITH MONOCLONAL ANTIBODY Aβ 1-11 IN TRANSGENIC MOUSE MODELS OF ALZEHIMER'S DISEASE. Esther S. Oh[1], Stina M. Tucker[1], Gay L. Rudow[1], Joanna L. Jankowsky[2], David R. Borchelt[1] and Juan C. Troncoso[1]*. [1]The Johns Hopkins University School of Medicine, Baltimore, MD. [2]California Institute of Technology, Pasadena, CA.

In the past several years, Aβ immunization has emerged as a potential tool for prevention and treatment of AD. Both immunization with Aβ peptide and passive transfer of Aβ antibody, also known as active and passive immunization respectively, are effective at reducing amyloid plaque burden. They also improve behavioral performances in transgenic (tg) mouse models of AD. Passive immunization seems especially promising as administration of mAb against Aβ does not seem to elicit a T-cell proliferative response to Aβ and its effects are self-limited by the life span of the antibody. We propose to determine whether peripheral administration of mAb Aβ 1-11 is effective in reducing Aβ plaques in newly developed tg mouse model CamKII-tTA x tetAPP/swe/ind with inducible APP expression. In order to determine that the effect of Aβ 1-11 is not limited to one tg mouse model, we will also be using another tg mouse model of AD, APP/PS1dE9. We will also be examining for evidence of mAb crossing blood brain barrier (BBB) and activation of microglia. Preliminarily, the kinetics study from APP/PS1dE9 shows that the plasma Aβ levels peak 1 day after mAb Aβ 1-11 adminstration. There is also immunohistochemical evidence that the mAb Aβ 1-11 crosses the blood brain barrier and activates microglia. Also, preliminary results from determining Aβ burden by stereology indicates that there is 38% decrease of Aβ burden in cortex and 27% decrease of Aβ burden in hippocampus in APP/PS1dE9 mice that have received 5 injections of Aβ 1-11.

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Aβ-IMMUNOTHERAPY IN A PRIMATE MODEL OF CEREBRAL β-AMYLOID ANGIOPATHY. Lary Walker*[1,2], Chris Ibegbu[1,3], Ron DeMattos[4], Yoland Smith[1,2], Hanie Elfenbein[1], Stephanie Stephens[1], Rebecca Rosen[1], Rolf Warzok[5], Mathias Jucker[6], Sam Gandy[7] and Harry LeVine[8]. [1]Yerkes Center; [2]Department of Neurology; and [3]Emory Vaccine Center, Emory University, Atlanta, GA. [4]Lilly Research Laboratories, Indianapolis, IN. [5]University of Greifswald, Germany. [6]University of Tuebingen, Germany. [7]Jefferson Medical College, Philadelphia, PA. [8]University of Kentucky, Lexington, KY.

Aβ-immunization therapy significantly reduces senile plaque load and improves behavioral performance in transgenic mice, but immunization in humans unexpectedly caused aseptic meningoencephalitis in a subset of patients. Preliminary findings also suggest augmented loss of brain substance in immunized humans. Available autopsy reports are consistent with encephalitis associated with cerebral β-amyloid angiopathy (CAA) and leukoaraiosis in two patients; in one of these cases, multiple cortical microhemorrhages occurred. The encephalitic and atrophic side-effects were not presaged by rodent immunization studies; hence, more biologically relevant models are needed to test Aβ-immunotherapies. Squirrel monkeys (*Saimiri spp*) are ideal subjects in that they develop Aβ-proteopathy beginning around 13 years of age, primarily in the form of CAA. We immunized three aged (18-23y) squirrel monkeys with recombinant, aggregated Aβ42, plus alum as adjuvant, at 0, 4 and 9 weeks; a control animal received scrambled peptide with alum. All three immunized monkeys developed evidence of a robust immune response to Aβ by 11 weeks. In this timeframe there was no indication of clearance of cerebral Aβ, and plasma Aβ levels were not significantly altered. In one Aβ-immunized subject, there were multiple cortical microhemorrhages, fibrinoid-like necrosis and focal inflammation associated with CAA, supporting the aged squirrel monkey as a biologically proximate model for assessing the safety and efficacy of Aβ-immunotherapy prior to human trials. The results also show the feasibility of generating a vigorous immune response to Aβ in aged primates using alum, a relatively safe, helper CD4 cell (T_H2)-biased adjuvant. Supported by the Woodruff Foundation and RR-00165.

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INVESTIGATION OF FC-RECEPTOR MEDIATED PHAGOCYTOSIS ON ABETA CLEARANCE *EX VIVO*. Stina M. Tucker, Esther S. Oh, Eduardo D. Zamora, Olga Pletnikova, Gay L. Rudow, David R. Borchelt and Juan C. Troncoso*. The Johns Hopkins University School of Medicine, Baltimore, MD.

Immunotherapy is a promising approach for the treatment of Alzheimer's disease (AD). Aβ immunization and passive administration of Aβ antibodies into transgenic mice has been shown to induce clearance of amyloid plaques as well as cognitive improvement. A human trial of Aβ vaccination in AD, which had to be stopped due to meningoencephalitis in 6% of the patients, provided some evidence for slower rates of cognitive decline. In order to develop effective and safe immunotherapy for AD, it is of utmost importance to determine the mechanisms by which immunotherapy induces Aβ clearance from the brain. Two mechanisms of Aβ clearance have been proposed; one advocating efflux of soluble Aβ from the brain to the blood upon antibody-Aβ binding in the periphery, another suggesting antibody-induced phagocytosis of Aβ by activated microglial cells. In the present study, we focus on the potential role of Fc receptor mediated phagocytosis by microglia in the clearance of CNS amyloid. FcγRIIB negatively regulates FcγRI and FcγRIII-induced phagocytosis by microglia as much as 30-fold. Therefore, we developed an *ex vivo* assay to examine whether FcγRIIB-deficient microglia phagocytose Aβ (in the presence of specific anti-Aβ antibodies) more efficiently than wild-type microglia. We also use this assay to screen the efficacy of various monoclonal Aβ antibodies in stimulating the phagocytosis of amyloid. Our goal in these experiments is to better understand the role of microglia in antibody-induced clearance of Aβ, and thereby contribute to the eventual development of a safe treatment for AD.

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CENTRAL NEUROCYTOMA (CN): A SERIES OF 45 PATIENTS BETWEEN 1971 AND 2003. Fausto J. Rodriguez, James L. Leenstra, Paul D. Brown, Hilary Blair, Bernd W. Scheithauer, Robert B. Jenkins, Steven E. Schild and Caterina Giannini*. Mayo Clinic College of Medicine, Rochester, MN.

CN are intraventricular neoplasms typically with favorable prognosis. This report comprises 45 histologically confirmed CN patients (25M, 20F) treated primarily with surgery and radiotherapy (RT). Median age at diagnosis was 28 years (range 5-57). We reassessed mitotic index (MI), presence of endothelial proliferation and/or necrosis in 36 available cases; MIB-1 labeling index by imaging analysis in 24; 1p/19q status by FISH in 19. Overall 5-year survival was 83% and local control (LC) was 65%. Removal attained was gross total (GTR) in 22 patients, and subtotal (STR) in 23. Five-year survival was 88% in GTR, 77% in STR. Postoperative RT enhanced LC at 5-years both in GTR (100% vs 43%, $p = 0.05$) and in STR (100% vs 50%, $p = 0.03$), but did not translate into a statistically significant survival benefit. Most CN showed benign histologic features, a minority demonstrating previously reported atypical features including MI ≥ 3 (9 of 36, 25%) and necrosis (6 of 36, 17%). MIB-1 was > 2 in 4 of 24 cases (17%). Statistical analysis is pending review of slides from 4 additional cases, which have been requested. FISH revealed intact 1p/19q status in 17 patients (2 technically failed). In summary, CN has favorable prognosis. Postoperative RT improves LC control. Atypical histologic features occur in a minority of CN. As previously reported, 1p/19q deletion, as seen in oligodendrogliomas, does not occur in CN further confirming their distinct nature.

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PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMAS HAVE AN IMMUNOPHENOTYPE TYPICAL OF THE CLINCALLY MORE AGGRESSIVE NON-GERMINAL CENTER DIFFUSE LARGE B CELL LYMPHOMA SUBTYPE. Amilcar A. Castellano-Sanchez[1]*, Arie Perry[2] and Richard Burack[1]. [1]Department of Pathology and Immunology, Division of Surgical Pathology, Section of Hematopathology, St. Louis, MO. [2]Division of Neuropathology, Washington University School of Medicine, Barnes-Jewish Hospital, St. Louis, MO.

By comparing molecular features of primary and secondary central nervous system lymphomas (P-CNSL and S-CNSL), we aimed to test whether P-CNSLs are best regarded simply as diffuse large B cell lymphomas (DLBCL) that happen to occur within the CNS or if they represent a distinct biological entity with a unique oncogenic mechanism. Clinically meaningful sub-classification of nodal DLBCL into germinal center-like (GC-like) and non-germinal center (non-GC-like) types has been demonstrated by expression microarray. An immunohistochemical panel (IRF-4/MUM1, Bcl-2, Bcl-6 and CD10) has shown to accurately predict this subtyping. To address the possible relationship between these DLBCL subtypes and CNS Lymphomas, we applied this panel to 27 examples of the latter. P-CNSL patients (16 total) had an average age of 58 with a 1:1 male to female ratio. S-CNSL patients (11 total) had a similar average age; the male to female ratio was ~2:1. All cases were large B-cell lymphomas. Results showed that 94% of P-CNSL were classifiable as non-GC-like, whereas only 50% of S-CNSL were non-GC-like, ($p = 0.023$ for 15/16 vs. 5/10 by Fisher's Exact test). The aggressive behavior of P-CNSL is often attributed to its location. However, our data suggest an additional reason for this behavior is that P-CNSL mimics the more aggressive form of nodal DLBCL. Furthermore, these data clearly identify a disparity between immunophentoypes of P-CNSL and some S-CNSL, indicating that P-CNSL may not simply be a group of DLBCL which happen to arise within the CNS, but are likely to be an inherently distinct biologic entity.

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CEREBRAL ERDHEIM-CHESTER DISEASE: THE CLINICOPATHOLOGIC SPECTRUM OF 5 CASES. Glenn D. Sandberg[1], Elisabeth J. Rushing[1], Iren Horkayne-Szakaly[1], Kelly K. Koeller[1], Metin Ozdermirli[2] and Charles S. Specht[1]*. [1]Armed Forces Institute of Pathology (AFIP), Washington, D.C. [2]Georgetown University Medical School, Washington, D.C.

Cerebral Erdheim-Chester disease is a rare non-Langerhans cell histiocytosis and its morphologic spectrum, biologic behavior, and factors influencing the latter are not clearly established in this location. Lesions may masquerade as other entities both clinically and pathologically; immunohistochemistry may aid in this differential. We studied the clinicopathologic features of 5 cases that included 4 male and a single female patient, ranging in age from 24 to 50 years of age (mean, 33 years). In one asymptomatic patient, multiple foci of bilateral low attenuation lesions were detected in CT scans obtained after a fall in the cerebral and cerebellar hemispheres. Another patient presented with a seizures and an intraparenchymal mass lesion. Morphologic evaluation revealed Touton-type giant cells and a mixed inflammatory background dominated by lymphocytes, occasional eosinophils and rare neutrophils. Immunohistochemical stains showed positivity for CD68 and absence of staining with CD1a. Immunolabeling with S100 protein was generally negative, although 2 cases revealed rare positive cells. Although ECD usually has distinctive morphologic features that facilitate the diagnosis; other patterns may occasionally predominate, causing confusion with various other histiocytic lesions. S100, CD1a, and CD68 represent the most helpful triad of immunomarkers serving to exclude Rosai-Dorfman disease and Langerhans cell histiocytosis, the two most common mimics.

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PRIMARY INTRACRANIAL GERMINOMA: A RETROSPECTIVE CLINICOPATHOLOGIC AND RADIOGRAPHIC STUDY OF 27 CASES WITH EMPHASIS ON CLINICAL OUTCOME. Eyas M. Hattab*, Kamnesh Pradhan and Annette C. Douglas-Akinwande. Indiana University Medical Center, Indianapolis, IN.

Primary germinomas of the central nervous system (CNS) are rare tumors of children and young adolescents in which imaging studies play an important role in their diagnosis. In this study, we analyze the clinical, radiographic and pathologic characteristics of 27 primary intracranial germinomas and pay special attention to their clinical outcome. Among the 27 cases examined, the male to female ratio was 4:1 in the pineal region but roughly 1:1 in the neurohypophyseal region. The median presenting age was 11 years with a range of 5 to 32 years. All germinomas were situated in the midline; exclusively in the pineal and neurohypophyseal regions, the latter being more than three times as common. Synchronous lesions were present in five patients. Radiographically, germinomas were well-circumscribed, lobulated lesions demonstrating mostly isointense signals on T1, T2, Flair, PD, and DWI sequences. They enhanced heterogeneously. 14 of 24 patients received chemotherapy (CT) and focal RT and of that cohort, 5 (36%) relapsed. In addition, 2 other patients who had received only CT also relapsed. Four relapsed patients were subsequently salvaged while two died of disease progression. No relapses were recorded in those patients who received CSI as a component of their initial therapy. **Conclusion:** Primary CNS germinomas typically present in the pineal or suprasellar region, sometimes synchronously. In our series, patients whose treatment regimen did not include CSI had poorer disease free survival outcome than expected for pure germinoma. Conforming to historical data highlighting the effectiveness of CSI, no patient treated with CSI relapsed.

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SCHWANNOMA HISTOLOGY REVISITED: CORRELATION OF HISTOLOGICAL FEATURES TO CLINICAL DIAGNOSIS OF SCHWANNOMATOSIS. Anat O. Stemmer-Rachamimov[1]*, P. Luigi. Poliani[5], Stacia DeSantis[3], Rebecca A. Betensky[3], Edward Hurwitz[1], Catherine L. Nutt[1,2] and Mia MacCollin[1,2]. [1]Massachusetts General Hospital, [2]Harvard Medical School, and [3]Harvard School of Public Health, Boston, MA. [4]University of Brescia, Brescia, Italy.

Schwannomas occur in three clinical settings with different prognosis and dissimilar genetic risks: as isolated sporadic tumors, in the setting of neurofibromatosis 2 (NF2) and in schwannomatosis. Patients with schwannomatosis have a remarkable propensity to pain, which remains unexplained. Early distinction of schwannomatosis from sporadic or NF2-associated schwannomas may be clinically challenging. **Objective:** To determine if schwannomas in schwannomatosis can be distinguished histologically from schwnannomas arising in other conditions and to define the tumors' unique histological characteristics. **Methods:** We analyzed 84 peripheral schwannomas previously characterized clinically and by molecular analysis, as sporadic, NF2- associated and schwannomatosis-associated tumors. We evaluated and quantitated the histological features of the tumors and the adjacent nerve. A pathologist (PLP), blinded to the patients' clinical diagnoses, predicted the diagnoses based on these features. **Results:** In a univariate analysis, the presence of nerve edema, entrapped intratumoral axons and intraneural growth pattern, were associated with the underlying diagnosis of schwannomatosis ($p = <0.00001$ for each). In addition, myxoid stroma, nerve edema, and intraneural growth pattern were also independent predictors of schwannomatosis in a multivariate model ($p = 0.007$, 0.039, 0.05, respectively). The sensitivity of the pathology diagnosis, using histology, to predict an underlying clinical diagnosis of schwannomatosis was 81%. **Conclusions:** We have identified pathological features unique to schwannomatosis, that, when present, distinguish schwannomatosis from non-schwannomatosis tumors and may aid in the early identification of these patients. In addition, these findings may provide clues to the unique predisposition of pain in schwannomatosis.

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AGE-DEPENDENT COGNITIVE IMPAIRMENT CORRELATES WITH CORTICAL ALPHA-SYNUCLEINOPATHY IN TRANSGENIC MICE. Manuela Neumann[1], Christian Freichel[2], Theresa Ballard[2], Laurence Ozmen[2], Edilio Borroni[2], Will Spooren[2], Philipp J. Kahle[3] and Hans A. Kretzschmar[1]*. [1]Center for Neuropathology and Prion Research, Munich, Germany. [2]F. Hoffmann-La Roche Ltd., Basel, Switzerland. [3]Laboratory for Alzheimer's and Parkinson's Disease Research, Munich, Germany.

Intraneuronal α-synuclein (αSYN) inclusions constitute the hallmark lesions of a number of neurodegenerative diseases, including Parkinson's disease and dementia with Lewy bodies. αSYN fibrillization has been shown to occur in aging transgenic animal models. To elucidate functional consequences of αSYN pathology *in vivo*, we have subjected transgenic mice expressing mutant [A30P]αSYN under control of the pan-neuronal Thy1 promoter and wild-type littermate controls to a behavioral test battery. Cognitive behavior was measured in a Morris water maze and in fear conditioning tests. At 4 months of age, transgenic mice behaved like controls. However, performance in the Morris water maze and fear conditioning were significantly impaired in (Thy1)-h[A30P]αSYN mice at 12 months of age. After completion of the cognition tests, the mice were sacrificed and the regional distribution of neuropathology examined by *in situ* labelling of proteinase K-resistant αSYN and silver staining. None of the 4 months old animals showed pathology. In contrast, 12 months old transgenic mice showed abundant PK-resistant αSYN in several brain regions, including the somatosensory cortex and the amygdala, especially the central nucleus. Thus, age-dependent cognitive decline of (Thy1)-h[A30P]αSYN mice correlates with the formation of pathological αSYN in cortical brain areas known to play a major role for neuronal circuits controlling emotion and memory. Therefore, these transgenic mice might be useful as a transgenic animal model for dementia with Lewy bodies.

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INTRANUCLEAR INCLUSIONS AND CYTOPLASMIC AGGREGATES IN TRANSGENIC MICE EXPRESSING A C-TERMINAL MUTANT OF THE FERRITIN LIGHT POLYPEPTIDE GENE. Ruben Vidal[1]*, Yingbin Su[1], Rose M. Richardson[1], Mario Widel[2], Leticia Miravalle[1] and Bernardino Ghetti[1]. [1]Indiana Alzheimer Disease Center and [2]Eli Lilly and Company, Indianapolis, IN.

Abnormal accumulation of ferritin and iron in the central nervous system (CNS) has been described in association with two autosomal dominant neurodegenerative diseases caused by mutations in the coding sequence of the *ferritin light chain* (*FTL*) gene. To study the role of the mutant ferritin polypeptide in the disease, we developed a transgenic (Tg) mouse model that over-express a human-FTL cDNA carrying the *FTL498-499InsTC* mutation under the control of the mouse Prion protein gene promoter. Control and FTL-Tg mice were studied neuropathologically, biochemically and behaviorally. Intranuclear and intracytoplasmic ferritin deposits were observed in both neurons and glia. Intranuclear and intracytoplasmic ferritin deposits were seen in neurons of the hippocampus, cortex, brainstem, cerebellum and spinal cord. Intranuclear ferritin deposits were also seen in cells of the choroidal epithelium. Ferritin deposits were also observed in cells of organs besides the CNS. Accumulation of SDS-insoluble ferritin was observed early in the course of the disease in the brain of 14 days old homozygous FTL-Tg mice. At age 4 months, homozygous mice showed a diminished capability to improve performance on the rotating rod. At age 8-9 months, hemizygous and homozygous animals show behavioral and motor deficits such as inability to spread hind limbs upon tail elevation, lack of grooming, and inability to grip a wire cage. These FTL-Tg mice recapitulate key features of the pathology observed in the human disease and may provide a valuable model for the study of neurodegeneration associated with ferritin accumulation. Supported by Alzheimer's Association, P30AG10133.

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CHARACTERIZATION OF A TRANSGENIC MOUSE EXPRESSING HUMAN P301S TAU. Anita G. Gnezda[1], Alexis Behm[1], John Greally[2], Nicholas Grahame[1], Bernardino Ghetti[1] and Jill R. Murrell[1]. [1]Indiana University School of Medicine, Indianapolis, IN. [2]Albert Einstein College of Medicine, Bronx, NY (Sponsored by Biagio Azzarelli).

Microtubule-associated protein tau is the major component of neurofibrillary lesions, a histopathological hallmark of many neurodegenerative diseases. Mutations in *MAPT* are associated with frontotemporal dementia and parkinsonism linked to chromosome 17. We are genetically, biochemically, neuropathologically, and behaviorally characterizing transgenic mice expressing human P301S tau. Southern blot analysis demonstrated integration of 60-70 copies of the transgene. FISH analysis mapped the transgene to the mouse X chromosome. Western blot analysis revealed soluble human tau expression as early as postnatal day seven. Insoluble tau was found in the cortex and spinal cord of one-month-old transgenic mice. Using phosphorylation-dependent anti-tau monoclonal antibodies, tau-immunopositive deposits were seen in neurons of the frontal cortex, piriform cortex, hippocampus, striatum, brain stem and spinal cord of 5 month-old animals. Glial cells also contained tau-immunopositive deposits. Analysis of younger animals is in progress. No cognitive deficits were found in 3, 5, and 7 month-old transgenics as evaluated by the Morris water maze test and instrumental learning reversal tasks at 8 months. By age 10-11 months, hemizygous mice showed motor and behavioral deficits such as absence of escape reflex on elevation by tail, inability to grasp a wire cage lid when inverted, hind limb dystonia and poor grooming. Starting at 1 month of age, mice are being tested to determine at which age diminished performance on a treadmill can be detected. This transgenic mouse may be a useful model to analyze the development of pathologic and behavioral abnormalities associated with a *MAPT* mutation. R01 NS37431, P30 AG10133.

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RNA-BINDING PROTEIN REGULATES EXPRESSION, ASSEMBLY AND AGGREGATION OF THE LIGHT NEUROFILAMENT (NF-L) SUBUNIT AND MAY HAVE A ROLE IN MEDIATING THE NEUROTOXIC EFFECTS OF UNTRANSLATED NF-L RNA ON MOTOR NEURONS OF TRANSGENIC MICE. W. W. Schlaepfer*, H. Lin, J. Zhai and R. Cañete-Soler. Division of Neuropathology, University of Pennsylvania Medical School, Philadelphia, PA.

We have shown that expression of a GFP reporter gene with NF-L RNA sequence in the 3'UTR leads to degeneration of motor neurons in transgenic mice and in cultured motor neurons. More recently, we have shown that the presence of a destabilizing element in the 3'UTR of a NF-L transgene causes aggregation of a GFP-tagged NF-L protein and loss of parent NF-L mRNA in transfected Neuro 2a cells. We now find that aggregation of NF-L protein is associated with co-aggregation of an RNA-binding protein (p190RhoGEF) that binds to the destabilizing element and stabilizes the NF-L transcript. Moreover, p190RhoGEF co-localizes with aggregates of GFP-tagged NF-L, but not with NF-L subunits that have assembled with NF-M or NF-H. These findings have led to a working hypothesis that interactions of p190RhoGEF with NF-L mRNA and with NF-L protein are part of a feedback pathway that down-regulates NF-L mRNA expression to the level of NF-L assembly with NF-M and/or NF-H. The working hypothesis is supported by additional studies, including co-transfection of NF-M with NF-L, siRNA-induced silencing of p190RhoGEF in transfected cells and examination of p190RhoGEF immunoreactivity in degenerating motor neuron degeneration of transgenic mice. Our working hypothesis provides a novel perspective on the steady state regulation of NF expression and the possibility that alterations of this feedback pathway may trigger degenerative changes in large NF-rich neurons.

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F-ACTIN CAPPING PROTEIN BETA SUBUNIT RNA INTERFERENCE CAUSES LOSS OF PROCESSES IN THE MAMMALIAN NEURONAL CELLS. Ivana Delalle*[1,2], Zhigang Zhou[1] and Li-Huei Tsai[1]. [1]Harvard Medical School and [2]Boston University School of Medicine, Boston, MA.

Intracellular inclusions and cytoskeletal effects are the hallmarks of the cellular degeneration. It is unclear whether these cellular abnormalities cause and/or contribute to degeneration or alternatively, are the consequence of degeneration. The FLP-FRT (FLP recombinase and FLP Recombination Target, FRT) system has been used to generate homozygous populations of wild-type and mutant cells in the Drosophila eye of an otherwise heterozygous fly so that effects of mutations on the development, growth, and viability of populations of cells could be studied in comparison with their wild-type neighbors. An interesting class of mutant representing mutations in the *Drosophila* orthologs of the F-actin capping protein beta and alpha subunit does not affect early cell division or cell viability, but the mutant tissue eventually dies with an accumulation of actin as the earliest abnormality detected (Delalle et al., 2005, submitted for publication). Concluding that degeneration can result directly from mutation in cytoskeletal regulators and cytoskeletal abnormalities, we have sought to explore the effects of loss –of-function of actin capping protein beta subunit (*Capzb*) using RNA interference (RNAi) in mammalian neuronal cells. 48-72 hrs after transfection with Capzb RNAi expression vector (p*Silencer*, Ambion) CAD cells (mouse neuroblastoma cell line) show downregulation of Capzb expression as demonstrated by Western Blot analysis accompanied by the loss of processes. The mechanism of this dramatic change in morphology is being investigated.

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A NOVEL SPIROPLASMA SP. RECOVERED IN CELL-FREE BROTH FROM SCRAPIE- AND CWD-INFECTED BRAINS VIA PASSAGE THROUGH EMBRYONATED EGGS. Frank O. Bastian*, Anthony S. Perry, Michael E. McDermott and Robert F. Garry. Tulane Health Science Center, New Orleans, LA.

Spiroplasma mirum, a wall-less bacterium, experimentally induces persistent brain infection in suckling rats characterized by spongiform encephalopathy. Spiroplasma ribosomal DNA has been demonstrated by polymerase chain reaction (PCR), Southern blot, and DNA sequence analysis in brains infected with transmissible spongiform encephalopathy (TSE) and not in normal brains. In this study, we set out to recover Spiroplasma in cell-free broth from scrapie-infected sheep brains and cervid brains infected with chronic wasting disease (CWD) via passage in embryonated eggs. Homogenates of scrapie- and CWD-infected brains in Sp-4 broth were filtered (220 nm) and inoculated into 8 day old embryonated eggs. Controls were inoculated with Sp-4 broth only. After incubation at 37ºC for 9 days, allantoic and amniotic fluids from each egg were inoculated into Sp-4 broth and incubated at 37ºC. At 16 days post-inoculation, the broths were examined by phase microscopy, and by electron microscopy. DNA extracts from the cultures were probed by PCR and DNA sequence analysis for Spiroplasma-specific ribosomal genes. Short motile helices were seen in the inoculated broth cultures by phase microscopy and as spiral wall-less bacteria (60 to 130 nm in diameter) by electron microscopy. PCR probes of the inoculated broth cultures revealed Spiroplasma-specific 16S rDNA 270 bp amplified PCR product which on DNA sequence analysis showed 98% homology with *S. mirum* but revealed nucleotide substitutions unlike any known Spiroplasma strain. In summary, we have recovered a novel Spiroplasma from scrapie- and CWD-infected brains tissues consistent with our hypothesis that Spiroplasma is involved in the pathogenesis of TSE.

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EXPERIMENTAL TRANMSISSION OF AN ATYPICAL FORM OF BOVINE SPONGIFORM ENCEPHALOPATHY. F. Tagliavini[1]*, R. Capobianco[1], C. Casalone[2], C. Miccolo[1], S. Suardi[1], M. Mangieri[1], L. Limido[1], M. G. Bruzzone[1], C. Corona[2], G. Zanusso[3], S. Monaco[3] and M. Caramelli[2]. [1]National Neurological Institute "Carlo Besta", Milano, Italy. [2]CEA-Istituto Zooprofilattico Sperimentale, Torino, Italy. [3]Department of Neurological and Visual Sciences, University of Verona, Italy.

A novel BSE phenotype has been recently identified in Italy. This phenotype (termed BASE) differs from typical BSE for the presence of PrP-amyloid plaques and the occurrence of a distinct PrP^{res} type, suggesting that BSE and BASE may be related to different prion strains. To investigate this issue we set up transmission experiments to a panel of four inbred mouse strains, including SJL, C57Bl/6, RIII and VM mice (n = 20 animals/group). At the time of writing, complete results are available for SJL and C57BL/6 mice. The SJL mice inoculated with BSE and vCJD have developed clinical signs of disease after 280±26 and 267±17 days, respectively, while SJL mice challenged with BASE were culled at 560 days post-inoculation without neurological symptoms. The incubation time of the C57BL/6 mice injected with BSE and vCJD was 445±42 and 411±62 days, respectively, while C57BL/6 challenged with BASE are still free of neurological symptoms. This clinical difference between groups was paralleled by a difference in the appearance of signal abnormalities at MRI. Neuropathological examination of BSE- and vCJD-infected mice sacrificed at the terminal stage of disease showed a spongiform encephalopathy with diffuse and plaque-like PrP^{res} deposits, and a PrP^{res} profile with a diglycosylated dominant pattern similar to that of BSE. By contrast, no PrP^{res} was detected in mice challenged with BASE. These data support the view that BSE and BASE are caused by prion strains with different biological properties. The "species barrier" to transmission of BASE seems to be substantially higher than that of BSE.

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EXPRESSION OF HERPES SIMPLEX VIRUS ENTRY RECEPTOR NECTIN-1 IN NORMAL AND NEOPLASTIC HUMAN NERVOUS SYSTEM TISSUES. Tibor Valyi-Nagy*, Grace Guzman, Stephen Oh, Herbert H. Engelhard and Deepak Shukla. University of Illinois at Chicago, College of Medicine, Chicago, IL.

Herpes simplex virus (HSV) is an important pathogen of the human nervous system and genetically modified HSV strains are promising vector candidates for gene and tumor therapies targeting the brain. Nectin-1 is an immunoglobulin-like adhesion molecule that participates in the formation of adherens junctions and synapses and serves as an entry receptor for HSV. To better understand the expression pattern of nectin-1 in the normal and neoplastic human nervous system, we have used immunohistochemistry to detect nectin-1 expression in normal adult human brain, spinal cord, trigeminal ganglia, and dorsal root ganglia (n = 10), and surgical specimens of nervous system neoplasms (n = 22) including diffuse astrocytoma (n = 2), anaplastic astrocytoma (n = 2), glioblastoma multiforme (n = 3), oligodendroglioma (n = 2), pilocytic astrocytoma (n = 1), pleomorphic xanthoastrocytoma (n = 2), ependymoma (n = 2), ganglioglioma (n = 1), meningioma (n = 3), medulloblastoma (n = 2), and schwannoma (n = 2). In the normal human nervous system, widespread nectin-1 immunoreactivity was detected in the soma and processes of central and peripheral nervous system neurons, in ependymal cells, choroid plexus epithelial cells, vascular endothelial cells and meningothelial cells. Oligodendrocytes, astrocytes, vascular smooth muscle cells, and Schwann cells showed variable immunoreactivity ranging from no to weak staining. Among tumors, schwannoma, fibrous meningioma, and medulloblastoma were nectin-1 negative. Oligodendroglioma, ependymoma, pilocytic astrocytoma, pleomorphic xanthoastrocytoma, diffuse astrocytoma, anaplastic astrocytoma, glioblastoma multiforme and meningothelial meningioma showed focal weak nectin-1 positivity. Ganglion cells of ganglioglioma were strongly positive. These studies may lead to a better understanding of cell targeting by HSV during HSV-induced neurological disease and in infections associated with HSV-based gene and tumor therapy.

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CHRONIC ACTIVE HERPES SIMPLEX ENCEPHALITIS IN AN ASYMPTOMATIC PEDIATRIC PATIENT: CASE REPORT. John E. Donahue, Cynthia Jackson, Qian Wu and William D. Brown. Rhode Island Hospital and Brown Medical School, Providence, RI.

A 9-year-old girl was found to have an abnormal CT scan after minor head trauma. She had a normal neurologic exam and no neurologic or academic complaints. MRI showed extensive bilateral hemispheric (R > L) areas of predominately white matter signal abnormality and a destructive process involving her right temporal lobe. Initial workup yielded normal CSF except for elevated CSF IgG synthesis index and quantitative CSF IgG levels. A maternal history of genital HSV infection and the patient's neonatal admission for focal seizures suggested the possibility of a herpes-related encephalitis or a postinfectious necrotizing autoimmune encephalitis. She was seropositive for HSV-1 (1:5120) and HSV-2 (1:10240). Stereotactic brain biopsy revealed chronic granulomatous encephalitis with numerous multinucleated giant cells, noncaseous necrosis, focal vasculitis, microcalcifications, and hemosiderin deposition. Immunostaining for pooled HSV antigens (type 1 and 2) was positive, as were two PCR probes for HSV protein segments. Serial MR imaging over the last year has shown marked reduction in signal abnormality in the face of daily treatment with oral valacyclovir and monthly intravenous steroid infusions. Imaging has shown apparent new extension of signal abnormality into the white matter of the left parietal lobe extending from the ependymal surface of the lateral ventricular posterior horn to the gray/white matter junction. The degree of contrast enhancement since presentation has improved. She continues to have a normal neurologic examination and no neurologic or academic complaints. We believe this to be the first reported case of an asymptomatic, chronic active herpes simplex encephalitis.

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BALAMUTHIA ENCEPHALITIS: AN EMERGING PATHOGEN IN IMMUNOCOMPETENT INDIVIDUALS? Abdelhalim Fouad[1], David Michelson[1], Fred Schuster[2], Carol Glaser[2], Nathaniel D. Wycliffe[1] and Ravi Raghavan[1]*. [1]Loma Linda University Medical Center, Loma Linda, CA. [2]California Department of Health Services, Viral and Rickettsial Disease Laboratory, Richmond, CA.

We report the fortuitous recognition of a rare form of encephalitis, a condition that often has fatal consequences. The patient is a 12 year old, immunocompetent Hispanic- American boy, who presented with symptoms of headache, fever and confusion. There was no significant past history of relevance. MRI indicated a large, 4.9 cm right occipital lesion with central enhancement, and bilateral smaller ill-defined lesions in the cerebral grey and white matter. The initial working diagnosis was acute disseminated encephalomyelitis. The boy responded to steroids briefly, but was readmitted 10 days later with multiple, bilateral ring-enhancing lesions, including the cerebellum. A brain biopsy revealed mainly focal perivascular lymphoplasmacytic infiltrates. Extensive work-up failed to reveal any conventional agents. Clinically the patient continued to be remarkably stable, and, in spite of involvement of multiple brain areas, did not have any focal neurologic deficits. A repeat biopsy revealed necrotizing chronic inflammation with abscess formation. Further investigation of serum and CSF samples revealed high antibody titers for a rare infectious agent, *Balamuthia mandrillaris*. Florid immunoreactivity for the organism in brain sections confirmed the serologic diagnosis. Balamuthiasis is a recently recognized infection caused by the free-living ameba, *B. mandrillaris*. The disease has an unusual predilection for Hispanic-American individuals, at least in California. The organisms have been reported in both immunocompromised and competent individuals. It is less fulminant than other amebic infections, but causes a more chronic, progressive encephalitis with potentially fatal consequences. However, rare individuals have survived the infection after timely intervention with multiple antimicrobial agents.

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MYOFIBER GROWTH AFTER REINNERVATION. Antje Bornemann and Zhe Zhou. Institute of Brain Research, University of Tuebingen, Germany (Sponsored by Hans Goebel).

Myofibers are able to recover from denervation atrophy when reinnervation takes place. It has been suggested that adult myoblasts (satellite cells) are activated after reinnervation to fuse with myofibers and to contribute to the myonuclear pool. We assessed the expression of transcripts in order to test the hypothesis that molecules regulating the cell cycle of satellite cells are upregulated after reinnervation. We denervated the right hindlimb of rats by lesioning the common peroneal nerve. We allowed the muscles to atrophy for 0-8 weeks before resuturing the cut ends microsurgically. The animals survived the resuturing step for 4-11 weeks. We conducted a microarray gene chip analysis to study the transcript expression of reinnervated tibialis anterior muscle when compared with the contralateral non-operated muscle. There was a significant downregulation of the transcript of α-amylase, an enzyme involved in glycogen degradation. There also was an increase of slow isoform of troponin C, myosin heavy chain polypeptide 7, rat heart myosin light chain 2 and calsequestrin 2. This demonstrates a shift of the reinnervated muscle towards the phenotype of a slow-twitch muscle. The results are in line with a possible increased energy demand by the growing myofibers, and with a partial cross-reinnervation taking place. There was no differential expression of transcripts involved in the regulation of the cell cycle. This suggests that activation of adult myoblasts satellite cells does not contribute to myofiber growth after reinnervation.

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LOBULATED / TRABECULAR CHANGE IN MYOFIBERS: A CRITICAL FINDING IN SOME FORMS OF DYSTROPHY. Philip J. Boyer*, Charlece S. Hughes, Sharon P. Nations, Gil I. Wolfe, M.D., Dennis Burns and Jaya R. Trivedi. University of Texas Southwestern Medical Center, Dallas, TX.

Background: Lobulated / trabecular change in myofibers, identified by trichrome stain and on oxidative enzyme histochemistry evaluation, an etiologically non-specific finding, has been identified in various forms of dystrophy and in other myopathic settings. **Objective:** We report two patients with lobulated / trabecular fibers as the only significant finding on muscle biopsy in whom a diagnosis of dystrophy was ultimately reached. **Methods:** Muscle biopsies from 49 and 61 year old men with 15 and 6 year histories of extremity weakness, respectively, and family histories of weakness were evaluated by an extended histologic and histochemical panel, by immunohistochemistry for a panel of dystrophin-associated glycoproteins, and by western blot for dysferlin and calpain. **Results:** In biopsies from both patients, in addition for mild neurogenic changes, the only significant histologic or histochemical abnormality was the presence of atrophy and lobulated / trabecular change in over 75% of type 1 myofibers. In both patients calpain expression was markedly reduced on western blot evaluation. Western blot and genetic testing to date are consistent with fascioscapulohumeral dystrophy and limb-girdle muscular dystrophy 2A, respectively. **Conclusions:** The presence of lobulated fibers, while non-specific, can be the only histologic or histochemical indicator of a dystrophy and warrants additional evaluation when indicated clinically. Dystrophies associated with this finding may be clinically and genetically heterogeneous. Deficient calpain expression can be primary or secondary.

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VESSEL CHANGES IN MALIGNANT HYPERTHERMIA SUSCEPTIBLE INDIVIDUALS. Marie-Magdeleine Ruchoux*, Catherine Godfraind*, Michel Franck, Thierry Depret and Renée Krivosic-Horber. Neuropathology, Lille Universitary Hospital, France.

Malignant hypothermia (MH) is a pharmacogenetic disorder with an autosomal inheritance. During exposure to triggering agents commonly used as halogenated anesthetics, affected individuals may develop a potentially fatal hypermetabolic syndrome caused by excessive calcium release from the sarcoplasmic reticulum in skeletal muscle. MH has been related to defects in the ryanodine gene receptor (RYR1 located in 19q13-1). Interestingly, the ryanodine receptor type 1 plays a crucial role in excitation-contraction coupling in skeletal muscle fiber, and it is responsible for releasing calcium ions from the sarcoplasmic reticulum. In skeletal muscle from MH susceptible individuals (MHS), some changes were described compared to skeletal muscle from non-MH susceptible relatives (MHN). On the other hand, no comment was done about skeletal muscle vessel walls. In this study, we reviewed the muscle biopsies of 3 families counting18 MHS individuals and their 17 MHN non-susceptible relatives) and observed the vessels. Ultra structural changes in vessel walls were systematically found in the MHS individuals and never found in their relatives. These changes included swollen reticulum and mitochondriae in endothelial cells, pericytes and vascular muscle cells and disorganization of pericyte and vascular muscle cell cytoskeleton. These new data suggest that vessels are likely to share some abnormalities with skeletal muscle fibers, which could also contribute to the disease.

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AXONAL DYSTROPHY AS A COMPONENT OF MULTI-EXPOSURE ORGANOPHOSPHATE-INDUCED DELAYED NEUROTOXICITY. B. S. Jortner*, S. K. Hancock, J. Hinckley, J. Carter and M. Ehrich. Laboratory for Neurotoxicity Studies, Virginia-Maryland College of Veterinary Medicine, Virginia Tech, Blacksburg, VA.

Axonal dystrophy is a dramatic form of nerve fiber degeneration, manifest by marked enlargement of affected neurites associated with aggregations of tubulovesicular elements, mitochondria and dense bodies (Jellinger, 1973). These lesions are seen in terminal regions of long central nervous system fibers in conditions such as aging, vitamin E deficiency, genetic disorders and some intoxications (Jellinger 1973; Ohara et al. 1995). In our study long-term exposure of rats to organophosphate neurotoxicants elicited a dramatic expression of axonal dystrophy. Long-Evans rats were administered two neurotoxic organophosphates in a setting of chronic stress over a 63-day period, with sacrifice on days 63 and 90 (after a 27 day exposure-free interval). The organophosphates were tri-*ortho*-tolyl phosphate (TOTP) given in 14 gavage doses of 75, 150 or 300 mg/kg and/or chlorpyrifos in two 60 mg/kg subcutaneous exposures. Corticosterone was added to the drinking water at 400 μg/ml to model chronic stress. The major neuropathologic change was distal gracile fasciculus and peripheral nerve Wallerian-like (axonopathy progressing to fiber breakdown) myelinated fiber degeneration, with smaller numbers of associated dystrophic axons (in gracile region only). This was time- and TOTP dose (at 300 and 150 mg/kg levels) -related. A dying-back pattern was seen with both types of fiber degeneration. In more proximal levels of the gracile fasciculus the dystrophic fibers often exceeded the Wallerian-like change. This suggests in this neurotoxic model, there is a dichotomous dying-back rate of these two axonal lesions, or that axonal dystrophy has a multifocal occurrence along the fiber. Supported by USAMRMC DAMD17-99-1-9489.

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NEUROPATHOLOGY OF VACUOLAR LEUKOENCEPHALOPATHY AFTER INHALTION OF HEROIN: REPORT OF THREE CASES. S. Yip, G. Medvedev, S. Spacey, G. R.W. Moore* and K. Dorovini-Zis*. Vancouver General Hospital, University of British Columbia, Vancouver, BC, Canada.

Inhalation of heated heroin vapour (heroin pyrolysate) or "chasing the dragon" is associated with a progressive leukoencephalopathy in a subset of users. Here we report the stereotypic clinical, neuroradiologic and neuropathologic features of this entity. Five cases of heroin-induced leukoencephalopathy were diagnosed at the Vancouver Hospital between 2002 and 2003. These patients were followed clinically and had serial MR imaging performed. Three patients died of their disease and had post-mortem examinations of their brains. All patients presented with varying degrees of bradykinesia, dysarthria, cerebellar dysfunction, lethargy and confusion. MR scans demonstrated extensive hyperintense T2 and FLAIR signal changes in the hemispheric white matter. The signal abnormalities also involved the cerebellar hemispheres and cerebral swelling was noted in all cases. Post-mortem neuropathological examination revealed diffuse, severe, vacuolar leukoencephalopathy involving the cerebral and cerebellar deep white matter. These changes were most pronounced in the occipital lobe and cerebellar white matter. Selective brain stem fiber tracts such as descending fibers, the superior cerebellar peduncles, the medial and lateral lemnisci and the medial longitudinal fasciculi were also involved. There was uniform sparing of the U-fibers. Axonal damage was also evident in the form of fragmentation and focal axonal spheroid formation in association with myelin vacuolation. Surprisingly, there was only a muted inflammatory response and mild gliosis. These cases of heroin-induced leukoencephalopathy underscore the unique features of this disorder which has diagnostic radiological and neuropathological findings.