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PL1
Evolving concepts of glioblastoma: 1863–2014
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The glioblastoma was well known, in the 1860s, to Virchow who appreciated the lesion’s infiltrative nature and relation to lower grade astrocytoma in some cases. Other seemingly more discrete types would, presumably, today be considered “primary.” It remained until 2008 for a molecular correlation, mutations in IDH1 or IDH2, to become clear. Later, Scherer was instrumental in refining the “secondary” subtype. Vascular proliferation was recognized and presciently presumed by Scherer to result from vasostimulatory factors released by the tumor. Decades later, Bevacizumab was created to block VEGF, unsuccessfully thus far insofar as overall survival is concerned. Discovery of tumor suppressor genes and oncogenes began with the first description of EGFR overexpression in 1984. Gains of chromosome 7 and losses of 10 were recognized at about the same time. The EGFRvIII variant was identified, and clinical attempts to target EGFR abnormalities continue today. Epigenetic influences on tumor development became apparent first in the role of MGMT and its interaction with effects alkylating agents. The pervasive influence of epigenetic mechanisms is increasingly appreciated. The role of micro RNAs, some of the latter with multiple targets, adds another layer of complexity. A lesion long known to be complex is thus even more so at closer and closer inspection. Glioblastomas now can be divided into subgroups on the basis of genetics, epigenetics, methylation profiles, micro RNAs, etc. Inter- and intratumoral heterogeneity is now well known. Recognition of therapeutic opportunities, and obstacles, continues to evolve.

PL2
The neuropathology of C9ORF72 mutations
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In 2011, abnormal expansion of a GGGGCC hexanucleotide repeat in a non-coding region of the chromosome 9 open reading frame 72 gene (C9ORF72) was identified as the most common genetic abnormality in familial and sporadic forms of frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) and the cause in most families where both conditions occur. The associated FTD phenotype is most often the behavioral variant and the motor neuron disease is usually classical ALS; however, a wide range of neurological features are now recognized, including aphasia, memory deficits, extrapyramidal dysfunction, psychosis and learning disability. The neuropathology of C9ORF72 mutation carriers includes a combination of frontotemporal lobar degeneration with TDP-43 immunoreactive (ir) neuronal and glial cytoplasmic inclusions and neurites (FTLD-TDP) and typical ALS with TDP-43-ir inclusions in motor neurons. The specific FTLD-TDP subtype is most often type B. Two additional pathological changes are highly characteristic of cases with the C9ORF72 mutation, each of which may play a pathogenic role. Aggregates of RNA, composed of the massively expanded GGGGCC repeat, can be demonstrated in neuronal nuclei using fluorescent in situ hybridization. These RNA foci are thought to bind and sequester specific RNA binding proteins, leading to the abnormal splicing of other genes. Another absolutely sensitive and specific pathological change is the presence of neuronal inclusions in the cerebellar granular layer, hippocampal pyramidal neurons and other neuroanatomical sites, that immunostain for markers of the ubiquitin proteasome system (i.e. ubiquitin and p62) but that are negative for TDP-43. It has recently been shown that these inclusions are composed of dipeptide repeat (DPR) proteins that result from the unconventional translation of the expanded GGGGCC repeats in both sense and antisense direction and in all reading frames (poly-GA, -GP, -GR and poly-PA, -PG, -PR respectively). Clinicopathological correlative studies have shown that the anatomical distribution of TDP-43 pathology correlates closely with the pattern of neurodegeneration and clinical phenotype. In contrast, the distribution of DPR pathology is highly consistent among cases, with no clinical correlation, suggesting that DPR inclusions may be a useful pathological marker for the presence of the C9ORF72 mutation but are of uncertain pathogenic significance.
C1
New concepts and classification of diffuse gliomas in adults
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The generic category of diffuse gliomas (DG) includes two distinct tumor entities: tumors derived from oligodendrocyte precursor cells – OPCs (OligoPDTs) versus those derived from stem cells (SCDTs).

Oligo PDTs are IDH-mutated; the tumor cells express the markers of OPCs PDGFRα and β. This category includes most WHO low-grade DG and comprises two variants: (1) tumors with TP53 mutations; (2) tumors with 1p/19q loss.

SCDTs are IDHwt, and contain CD34+ tumor stem-like cells (TSC). This category includes most WHO high-grade DG and includes two variants: (1) malignant glioneuronal tumors (MGNs) that express markers of glial and neuronal lineage; (2) glioblastomas that according to our restricted definition (GBrd) express markers of glial lineage exclusively and presence of necrosis is required for the diagnosis.

In our series of patients with a retrospective diagnosis of OligoPDT, MGNt and GBrd, the median age was, respectively, 40, 57 and 65 years (p<0.0001), median overall survival (OS) was 12, 1.3, and 0.5 years (p<0.0001); OS in OligoPDTs with or without p53 accumulation: 7.7 vs. 18.1 years (p<0.0001).

The behavior of OligoPDTs and SCDTs is similar to that of adult OPCs from which they derive. OligoPDTs initially grow as isolated tumor cells – ITCs (50% of the cases at the time of diagnosis) (i) as astrocytes during development and reactive astrocytes in the injured brain, stromal astrocytes induce microangiogenesis and secrete PDGF, which induces the proliferation of OPCs, and block their differentiation. (ii) In contrast to adult OPCs originating in the cortex, those developing in the sub-ventricular zone (SVZ) migrate long distances within the white matter: on MRI, OligoPDTs with a superficial localization are typically well circumscribed; those located in the white matter show an infiltrative pattern and have a worse prognosis (OS: 18.1 vs. 5.4 years), (iii) lactate dehydrogenase (LDH), is not expressed in the oligodendrogial population of the adult brain and in tumor cells of OligoPDTs suggesting that IDH mutation allows OligoPDTs to overcome the silencing of LDH. SCDTs have an autonomous growth and are highly angiogenic. It has been reported that, as their normal counterpart, TSC generate endothelial cells and that the pluripotency of stem-cells decreases with age: GBrds arise in elderly patients, do not express markers of neuronal lineage and PDGFR-negative GBrds carry a worse prognosis (OS: 5.9 vs. 9.7 months, p=0.013).

C2
Frontotemporal dementia
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The last decade has seen both an expansion and transformation in our knowledge of the genetic etiologies and the molecular pathologies associated with frontotemporal lobar degeneration (FTLD), which encompasses a clinically and neuropathologically heterogeneous group of frontotemporal diseases. Increasingly, there is evidence of a genetic, neuropathological and clinical overlap between both FTLD and amyotrophic lateral sclerosis/motor neuron disease (ALS/MND). Most frontotemporal diseases are characterized by the pathological aggregation of misfolded proteins, either in neurons or glial cells, or both. Specific diagnosis of disease within the broad group of frontotemporal diseases now requires immunohistochemistry to determine the molecular pathology, morphology, and distribution of lesions in the neuraxis, and thereby identify the neurodegenerative disease.

About one half of cases with frontotemporal disease are characterized by inclusion bodies containing the transactive response (TAR) DNA-binding protein of 43 kDa (FTLD-TDP). This molecular pathology is found in sporadic cases of frontotemporal disease as well as those with autosomal dominant FTLD/ALS with C9orf72, GRN, VCP, or TARDBP gene mutations. The neuropathology of these conditions is generally characterized by ubiquitin/p62 and TDP-43-positive neuronal cytoplasmic inclusions (NCIs), neuronal intranuclear inclusions (NIIs), dystrophic neurites (DNs), and glial cytoplasmic inclusions (GCIs), but the distribution and density of lesions may vary between subtypes.

Although mutations in the FUS gene are most frequent in familial ALS, three apparently sporadic diseases have distinct FUS proteinopathy (FTLD-FUS): neuronal intermediate filament inclusion disease (NIFID), basophilic inclusion body disease (BIBD), and atypical FTLD with ubiquitin-immunoreactive inclusions (aFTLD-U).

About one half of cases have abnormal intracellular cytoplasmic accumulations of the microtubule-associated protein (MAP) tau encoded by MAPT, which include neuronal intermediate filament inclusion disease (NIFID), progressive supranuclear palsy (PSP) (4R), and others, and familial cases caused by mutations in MAPT.

Recent developments in the molecular pathology and genetics of FTLD now dictate that a minimal panel of pathological investigations is required for correct diagnosis in this group of diseases. Standardization of nomenclature and approach will facilitate better understanding of clinico-pathologic correlations, provide insights into pathogenesis, and guide the construction and validation of in vivo models.

C3
Protein aggregate myopathies
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Intra- and extracellular protein aggregation is a well-known principle in neuropathological conditions, e.g. Alzheimer and Parkinson diseases, giant axonal neuropathy and myofibrillar myopathies (MFM). In myopathology, protein aggregation may be part of lesions, such as ragged red fibers or cores, encountered in certain conditions, e.g. distal myopathies, congenital myopathies, and inclusion body myositis, and foremost in myofibrillar myopathies, the true protein aggregates myopathies (PAM). Among these, primary ones, i.e. those with accretion of mutant proteins, e.g. desminopathies, zaspopathies and others may be distinguished from secondary ones where a mutant protein may not be present. The true frequency of secondary MFM/PAM is not precisely known. Together with the mutant protein a multitude of other extralysosomal proteins may accrue. PAM or MFM are late onset distal myopathies of autosomal-dominant inheritance, in certain forms associated with cardiomyopathy, and protein aggregates, which show granulofilamentous material by electron microscopy in desminopathies and alpha B crystallinopathies and destruction of sarcomeres in all forms of MFM. Frequent rimmed vacuoles tie protein aggregation to autophagy. Recently autosomal-recessive forms have been described in desminopathy and alpha B crystallinopathy. From these PAM marked by impaired cytosolic degradation, catabolic forms, anabolic forms owing to impaired synthesis and maturation may be distinguished, represented by actin filament aggregation myo-
pathy, a form of nemaline myopathy, and myosin storage/hyaline body myopathy, often affecting young individuals with heterozygous mutations in the respective ACTA1 and MYH7 genes.

S1: Updates in neurodegenerative diseases
Update in amyotrophic lateral sclerosis: an experience from Japan
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One of the most interesting things for the unique features in amyotrophic lateral sclerosis (ALS) is that the frequency of the patients with c9FTD/ALS is extremely low in Japanese population. The diseases resulting from a G4C2hexanucleotide repeat expansion in C9ORF72 occurs on a chromosome 9p21 locus are referred to as ‘c9FTD/ALS’. The c9FTD/ALS is 27–46% in FALS and 2–8% in SALS in whites. The presence of a risk haplotype suggests that c9FTD/ALS is resulted from a single founder mutation or a risk haplotype descending from a Scandinavian ancestor. In Japanese, the frequencies of c9FTD/ALS are 3.6% in FALS and 0.1% in SALS. The estimated haplotype indicated that these patients also have the risk haplotype identified in whites. Neuropathological findings of an autopsy case with c9FTD/ALS were indistinguishable from those of white patients. In clinical features, the frequency of dementia is low, and none of the familial cases show vertical transmission in Japanese c9FTD/ALS. The molecular link between the C4G2 expansion and TDP-43 pathology may provide new insight for the mechanism of the sporadic ALS associated with TDP-43 pathology.

Progressive supranuclear palsy and corticobasal degeneration
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PSP and CBD are major sporadic 4-repeat tauopathies. Tufted astrocytes (TAs) in PSP and astrocytic plaques (APs) in CBD have been regarded as the characteristic pathological hallmarks. To better outline the pathological features of each disease and define the astrocytic inclusions, we reviewed 95 PSP cases and 30 CBD cases. The pathological features of PSP are divided into three representative subtypes: typical PSP type, pallido-nigro-luysian type (PNL type), and CBD-like type. CBD is divided into three pathological subtypes: typical CBD type, basal ganglia-predominant type, and PSP-like type. TAs are found exclusively in PSP, while APs are exclusive to CBD, regardless of the pathological subtypes, although some morphological variations exist, especially with regard to TAs. The overlap of the pathological distribution of PSP and CBD makes their clinical diagnosis complicated, although the presence of TAs and APs differentiate these two diseases. Immunoelectron microscopic examination using quantum dot nanocrystals revealed filamentous tau accumulation of APs located in the immediate vicinity of the synaptic structures, which suggested synaptic dysfunction by APs. The characteristics of tau accumulation in both neurons and glia suggest a different underlying mechanism with regard to the sites of tau aggregation and fibril formation between PSP and CBD: proximal-dominant aggregation of TAs and formation of filamentous NFTs in PSP in contrast to the distal-dominant aggregation of APs and formation of less filamentous pretangles in CBD. The pathological subtypes of PSP and CBD suggest that the clinical phenotypes are in accordance with the pathological distribution and degenerative changes.

Synucleinopathy: beyond the brain
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Concepts regarding the pathogenesis of Parkinson’s disease (PD) have undergone substantial revision over the past decade. The Braak hypothesis has engendered widespread support for a spatiotemporally dynamic model whereby alpha-synuclein (AS) aggregation is initiated not in the substantia nigra, or even the central nervous system, but in the peripheral nervous system from which it “spreads” over time to ultimately involve multiple CNS loci. Initial AS aggregation may be induced by some yet-to-be identified environmentally derived toxin or microbial pathogen. This novel model has crucial pathogenic, diagnostic, and therapeutic implications. The olfactory mucosa and the enteric nervous system (ENS) have been specifically implicated as initial sites of AS aggregation. Within the latter, there appears to be a rostrocaudal gradient of decreasing Lewy pathology, suggesting differential vulnerability of distinct levels of the gastrointestinal tract to AS aggregation. Our studies of the topographical distribution of AS in the ENS of neurologically intact humans have revealed that the mucosal plexus of the vermiliform appendix is particularly enriched in AS. This, combined with its dense innervation from neurons in the dorsal vagal complex, one of the first CNS sites to be affected by Lewy pathology, render the appendical ENS an attractive candidate as a locus for initial AS aggregation. The status of the appendix as an “immune organ” has further pathogenetic implications as there is increasing support for a role for immune mechanisms in PD pathophysiology. In this context, a hypothesis regarding a possible role for Epstein–Barr virus-induced autoimmunity in PD pathogenesis will be discussed.

Characterization of globular glial tauopathies
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Widespread presence of globular glial inclusions (GGIs) characterizes a group of 4-repeat (4R) tauopathies. Tau immunohistochemistry reveals 4R immunoreactive globular oligodendroglial and astrocytic inclusions. The oligodendroglial inclusions are Gallyas positive; however, the astroglial inclusions are predominantly negative for Gallyas silver staining. The oligodendroglial tau-positive globular inclusions are reminiscent of the alpha-synuclein positive oligodendroglial cytoplasmic inclusions seen in multiple system atrophy. Presence of GGIs associates with a range of clinical presentations, which correlate with the severity and distribution of underlying tau pathology and neurodegeneration. The diverse clinicopathological presentations and GGIs have been described in the literature using various terminologies. A group of neuropathologists formed a consensus on terminology to classify these cases. We agreed that GGIs were present in all the cases reviewed and that the morphology of globular astrocytic inclusions was different to tufted-astrocyte type inclusions. The different morphological subtypes are likely to represent a spectrum of a distinct disease entity. The term globular glial tauopathy (GGT) was proposed. Three major types were distinguished. Type I cases typically present with frontotemporal dementia, type II cases are characterized by pyramidal features reflecting corticospinal tract degeneration and type III cases can present with a combination of frontotemporal dementia and motor neuron disease with or without extrapyramidal symptoms. Significant degeneration of the white matter is a feature of all GGT subtypes. Improved detection and classification of GGIs helps future studies on tauopathies.

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Advances in understanding the neuropathology of multiple system atrophy
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Multiple system atrophy (MSA) is a sporadic adult onset neurodegenerative disease with clinical features including parkinsonism, cerebellar ataxia and autonomic failure. Neuropathological examination shows regional neuronal loss and gliosis with widespread oligodendroglia α-synuclein immunoreactive glial cytoplasmic inclusions (GCIs), which are considered to be the hallmark of MSA. α-Synuclein immunoreactive inclusions are also found in neurons and MSA is regarded as a member of the group of α-synucleinopathies that also includes idiopathic Parkinson’s disease and dementia with Lewy bodies. The pattern of regional neuronal loss has given rise to the concept of pathological sub-types of MSA and the prevalence of these sub-types has been shown to vary between different ethnic populations. GCIs are considered to play a key role in the pathogenesis of MSA as their density in affected brain regions correlates with neuronal loss and disease duration. To date there is limited understanding of the mechanisms leading to GCI formation and this is a focus of current research. In this presentation, the neuropathological features of MSA will be described and current insights into the disease pathogenesis will be discussed.

S2: Microvascular neuropathology

Introduction and classification of small vessel disease
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Central nervous system (CNS) small vessel disease is an arbitrary subdivision that separates those groups of disorders that affect the nervous system from injury to vessels outside the parenchyma of the brain, i.e. large extra cranial vessels that supply the nervous system and intracranial vessels within the subarachnoid space, from those that penetrate the parenchyma of the brain, i.e. typically arterioles, venules and the components of the microcirculation. This operational definition serves to distinguish major pathophysiological mechanisms of stroke and corresponding clinical syndromes that differ greatly between the two groups.

Three generalizations apply to most forms of CNS small vessel disease – these are disorders that affect multiple vessels throughout the neuraxis; a broad range of abnormalities accompanies the vascular injury, be it inflammatory, or predominantly non-inflammatory; the vascular lesions are associated either with focal hemorrhage or thrombosis, with corresponding parenchymal brain injury. In some, the principal clinical manifestations are those of focal neurological deficits referable to the anatomic region involved, whereas in others, syndromes of dementia (with or without associated degenerative disease), or encephalopathies, predominate. The armamentarium of new radiological imaging methods has enhanced awareness of these important causes of neurological morbidity. Some of these affect predominantly or exclusively the CNS, whereas others are systemic vascular disorders that involve the brain and spinal cord along with other organ systems. For purposes of classification, and conceptually, these are disorders of the “microcirculation” and small blood vessels in general. The so-called “small arteries” or arterioles include perforators with diameters from 40 to 400μm. Some emerge from the leptomeningeal arteries, enter the brain parenchyma from the surface of the brain, and extend within to a variable depth, some reaching the deep white matter as medullary branches. Other perforators enter the brain at the base (supplying the basal ganglia and thalamus), and yet others irrigate the brainstem arising from long and short circumferential branches. For the most part, these are end-arteries with limited collateral anastomoses until the capillary network is reached.

The classification below is offered as a framework for the understanding of CNS small vessel disease:

A. Systemic diseases with CNS involvement
   1. Atherosclerosis, diabetes, hypertension – lacunar infarction
   2. Cerebrovascular complications of hematologic disorders – hemorrhage/infarction
      a. Coagulopathies and occlusive disease, e.g. hyperviscosity syndromes, cryoglobulinemia, TTP, DIC
      b. Hemorrhagic diatheses, e.g. drug-induced, myeloproliferative
   3. Arteriopathic leukoencephalopathy, i.e.Binswanger disease

B. Primarily “inflammatory” diseases with systemic involvement
   1. Infectious: e.g. fungal (Aspergillus), bacterial, (TB), viral (CMV)
   2. Non-infectious
      Associated with “connective tissue diseases”
      a. Polyarteritis nodosa
      b. Hypersensitivity angiitis (Churg and Strauss)
      c. Systemic lupus erythematosus
      d. Sjögren syndrome
      e. Wegner’s granulomatosis
      Associated mainly with systemic dermatologic and mucocutaneous syndromes
      1. Behcet’s disease
      2. Sneddon syndrome
      3. Köhlmeier-Degos disease
   
C. Non-infectious (idiopathic) selectively (or predominantly) CNS small blood vessels

Inflammatory
   1. Primary CNS granulomatous angiitis (with or without amyloid angiopathy)
   2. CNS “Autoimmune Vasculitis” in children or adults
   3. Subacute diencephalic angioencephalopathy (possibly related to AV fistulas)

Non-inflammatory
   1. Hereditary and non-hereditary amyloid angiopathy
   2. CADASIL

D. Other
   1. Systemic mitochondrial disorders affecting CNS blood vessels, e.g. MELAS
   2. Systemic vasculopathy associated with cancer

For this mini-symposium, we have selected only a few topics for in-depth discussion:
   1. Introduction to symposium – basic concepts and classification (De Girolami)
   2. Neuroradiologic considerations (Gasparetto)
   3. Hereditary diseases of small vessels: Genetics (Tourrier-Lasserre)
   4. Hereditary diseases of small vessels: Pathology (Gray)

Neuro-radiologic considerations

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In this presentation, we will discuss the most common neuroimaging findings in patients with microvascular pathology. First, we will describe the imaging patterns of microvascular pathology in each magnetic resonance imaging sequence (i.e. T1-WI, T2-WI, FLAIR, etc.).
Then, we will discuss the differential diagnosis that has to be considered when reading cases that present a “microvascular pathology” imaging pattern. Finally, we will assess the role of the advanced magnetic resonance imaging techniques in the evaluation of patients with microvascular pathology.

Hereditary diseases of small vessels
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Neuropathological data are available only in a few cases of non-amyloid hereditary diseases of CSV and are occasionally diagnostic. CADASIL is the prototype of dominant hereditary diseases of CSV. Since the initial description (Baudrimont et al. Stroke 1993), its neuropathology has been established by numerous reports. Changes in CSV are diffuse and include specific alterations: granular basophilic deposits presenting at e.m. as granular osmiophilic material “GOM” and accumulation of the extracellular domain of Notch 3 (ECDN3), which can be identified by immunohistochemistry (Joutel et al., J Clin Invest 2000). These diagnostic changes are systemic and can be observed on skin biopsies. Parenchymal brain changes are considered the consequence of ischemia due to vascular stenosis, which is a feature of CADASIL. Subcortical lacunar infarcts and arteriopathic leukoencephalopathy are characteristics of the disease and included in the eponym. Frequent, less classical changes: status cribrosus and involvement of the cerebral cortex, which may result from different mechanisms, will also be discussed.

In the other dominant hereditary diseases of CSV, neuropathological data are rare and less specific. Retinal vasculopathy with cerebral leukodystrophy is characterized by involvement of SV of the brain and retina. Distinctive multilaminated vascular basement membranes in systemic organs have been found at e.m. However, neuropathological studies are few and non-specific. COL 4A1 and COL 4A2 mutations cause systemic small vessel disease. Involvement of the vascular basal membrane in systemic organs has been shown at e.m. but neuropathological study has only been performed in fetuses. It showed cavitary necrotic lesion (porencephaly) and old and recent hemorrhages with abnormalities of small vessel walls.

Idiopathic basal ganglia calcification or Fahr disease is a common incidental neuroimaging or post mortem finding. In some cases, it has been linked to mutations in PDGFβR and in PDGFβ and experimental studies suggest that the calcium deposition depends on the loss of endothelial PDGF-B and correlates with the degree of pericyte and blood-brain barrier deficiency.

Signal transducer and activator of transcription 3 (STAT3) deficiency is a rare primary immunodeficiency referred to as an autosomal dominant hyper-IgE syndrome. Recently, vascular abnormalities in heart and brains have been described radiologically. Postmortem examination of a patient who died from ruptured basilar artery aneurysm also showed vascular leukoencephalopathy and abnormalities of CSV likely to result from connective tissue disease.

In a single case of hereditary extensive vascular leukoencephalopathy (HEVL) mapping to chromosome 20q13, neuropathological study showed very specific changes of small terminal arterioles and vasa vasorum, more severe in the cortico-subcortical areas, basal ganglia, and subependymal regions.

In Swedish hereditary multi-infarct dementia as in pontine autosomal dominant microangiopathy and leukoencephalopathy (PADMAL), the gene mutation has not been identified. Neuropathological changes are comparable and include multiple lacunar infarcts with severe involvement of the pons. CSV changes include concentric intimal proliferation with hyperelasticity and atrophy of the tunica media in the absence of GOM or ECDN3 deposits.

Recessive hereditary diseases of CSV include CARASIL in which the gene mutation has been identified and neuropathological studies have revealed variable, usually non-specific, changes of CSV, and leukoencephalopathy, cerebral calcifications and cysts (LCC) the responsible gene mutation of which is not identified but the hereditary character of which is likely given its very suggestive pathology and a notion of consanguinity in some families.

Neuropathogenesis of enterovirus 71 infection
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Enterovirus 71 (EV71) (family Picornaviridae, genus Human Enterovirus A) belongs to a large group of enteroviruses that also includes the poliovirus. It usually causes mild paediatric hand, foot and mouth disease, manifesting as skin rashes or vesicles in these classical sites. More rarely, complications such as meningitis, acute flaccid paralysis, transverse myelitis and encephalomyelitis may occur. Huge outbreaks of EV71 infection with significant central nervous system (CNS) complications in the Asia-Pacific region and beyond have become major public health concerns since anti-viral drugs and vaccines are not yet available. Autopsy studies of fatal encephalomyelitis showed a stereotyped topographic distribution of inflammation, viral infection and destruction of neurons mainly in the spinal cord, brainstem, hypothalamus and cerebellar dentate nucleus with minimal inflammation in the cerebral hemisphere, including the motor area. Entry into the CNS is believed to be via a retrograde peripheral motor nerve route where spread within the CNS may involve other neuronal pathways. Recent evidence suggests that viral replication could occur in the tonsillar crypt epithelium, thus facilitating oral and faecal viral shedding. The tonsil may also be a portal for viral entry into the body. Presumably this is followed by viral replication in other extra-CNS sites (e.g. skin) and/or viraemia, leading to neuroinvasion in susceptible patients. A hypothesis for EV71 transmission in the CNS will be discussed and compared with poliovirus and Japanese encephalitis virus. The role of the Scavenger Receptor B2 receptor in EV71 neurotropism will be reviewed.

New infections like the expanding JCV neuropathology
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Recent years have seen the emergence of new viral threats like SARS or MERS coronaviruses, Ebola, dengue, West Nile fever, avian flu, or Hanta, Nipah or Hendra. Most of these infections potentially affect the nervous system and thus are of relevance for neuropathology. However, even hitherto well-known viruses may change their host spectrum, adapt to changed environments, or change their tropism, leading to new and unrecognized diseases or therapy-related complications.

One paramount example is the JC polyomavirus (JCV), the causative agent of progressive multifocal leukoencephalopathy (PML) known since 1971. Despite high seroprevalence, JCV has been pathogenic only in immune compromised conditions including AIDS. More recently, PML has emerged as rare but important complication of MS therapy with monoclonal antibodies like natalizumab or rituximab. Moreover, efficient therapy of the underlying immune deficiency, e.g. by HAART in AIDS, may result in the immune reconstitution inflammatory syndrome (IRIS) that converts PML into a fulminant inflammatory disease. Finally, the spectrum of JCV tropism has expanded to meninges, cortical neurons and cerebellar granule cells, giving rise to new diseases of JCV meningitis/encephalopathy or JCV granule cell neuronopathy (JCV-GCN). The neuropathological and immune basis of the first case of JCV-GCN after natalizumab treatment, complicated by IRIS, is discussed.
Rabies encephalitis
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The pathogenesis of furious and paralytic rabies remains to be elucidated. Previous studies in humans failed to identify differences between the two clinical forms, and this could be due to the methodology and/or examination late in the disease course. To observe early changes, diffusion tensor imaging (DTI) technique was used to assess blood brain barrier (BBB) (mean diffusivity, MD) and tract integrity (fractional anisotropy, FA) of rabid dogs. Preserved BBB was noted in both clinical forms, whereas cytotoxic edema was more pronounced in the paralytic rabies. Impaired FA (disrupted neural tract integrity) was found at the brainstem of paralytic dogs. At postmortem, more rabies viral antigen was detected in the furious as compared to paralytic rabies in many of the central nervous system regions. Caudal-rostral polarity of rabies viral antigen as observed in both clinical forms, in order from greatest to least: spinal cord, brainstem, cerebellum, midline structures, hippocampus, and cerebrum. Viral RNA level was significantly higher in the cerebral cortex, thalamus, and brainstem of dogs with the furious sign. Of particular interest, a striking inflammatory response was observed in both clinical forms, and this finding correlated well with the altered neural tract integrity and presence of interleukin-1β and interferon-γ mRNAs. Disruption of axoplasmic flow by inflammation in the brainstem could potentially retard viral propagation toward the cerebral hemispheres in the case of paralytic rabies, and explain the longer survival period as compared to the furious counterpart.

Recent findings in neuropathology and neuroradiology of HIV infection
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In order to achieve the changes related to the regular use of the highly active antiretroviral therapy (HAART), we present the spectrum of the neuropathology findings and the correlation between the CNS MRI scans prior to patients’ death and the neuropathological features as well as clinical-laboratorial data from 70 HIV-infected adult patients, who were followed at Hospital of Clinics of Ribeirão Preto School of Medicine at University of São Paulo. After autopsy, the brains were fixed in 10% formalin for 2 weeks before cutting and representative areas were sampled according to standard protocol. Demographic, clinical, and laboratory data were recorded from clinical charts. As expected, we found a high rate of irregular use of HAART in patients with clinical deterioration, but the use of HAART, even irregularly, was associated with longer patient survival. However, the frequency of CNS infections and/or cognitive impairment was significantly higher in patients who did not receive any HAART therapy. The most frequent CNS opportunistic agents were T. gondii, C. neoformans (leptomeningeal dissemination, cryptococcomas and basal ganglia “soap bubbles”), Mycobacterium tuberculosis (leptomeningitis) and cytomegalovirus. The most relevant finding was more than one infectious agent in the same brain or cases with multiple old lesions (“infection scars”). Interestingly, we had some cases with concomitant primary CNS lymphoma, HIV-leukoencephalopathy, PML and nodular encephalitis. Despite some caveats, the best correlation between MRI and histopathology was seen in foci of coagulative necrosis in toxoplasmosis, with eccentric target sign and T1 hyperintensity on lesions with calcification.

The emerging viral encephalitides
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Despite major advances in the control of infectious diseases, new pathogen/human host associations continue to emerge. Historically, many of these emergent infections were the result of disruption of biosphere equilibriums with introduction of new pathogens into previously unexposed populations. For unknown reasons, infection of the brain has suffered a disproportionate share of these diseases. In addition to ecological disruptions, man has chosen to deliberately manipulate pathogens to achieve lethal destruction of his fellow man. These bioterrorism efforts have led to introduction of well-characterized agents through novel routes (e.g. aerosol dispersion) circumventing normally protective immunological responses. To protect himself from infectious agents, man has also attempted to created vaccines to ovlate severe disease associated with initial exposure. While on the whole, these vaccines have been highly successful means of conferring protective immunity, they do not provide as robust an immune protection as a natural infection. Unfortunately, protection from lethal systemic disease has not been universally associated with protection from lethal encephalitis. Finally, the sheer size of the human population and its associated biome have resulted in the emergence of virulent pathogenic strains from ubiquitous less pathogenic organisms. Again, for unclear reasons, many of these new pathogens exact a profound toll on the human brain.

MicroRNA regulation of progressive multifocal leukoencephalopathy
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Progressive Multifocal Leukoencephalopathy (PML) is a fatal disease of the CNS, result of the opportunistic infection by the human neurotropic JC virus (JCV), and frequently seen in immunocompromised patients. PML is characterized by extensive demyelination of the subcortical white matter caused by the lytic destruction of oligodendrocytes. There is no effective treatment for PML and the median survival rarely surpasses 6 months. A growing concern is the development of PML as side effect of immunomodulatory therapy in the treatment of numerous autoimmune diseases. MicroRNAs are small (~22 nucleotides), non-coding RNA molecules that are present in the genomes of almost every organism and influence biological processes by regulating expression of their target gene(s). It is predicted that approximately 50% of all genes are regulated by microRNAs making them important regulators of most physiological processes. MicroRNAs making them important regulators of most physiological processes, including apoptosis, differentiation, and immune regulation; as well as many pathophysiological processes such as inflammation, neurodegeneration, and cancer. Preliminary data of the first high-throughput microRNA profile of PML revealed 54 microRNAs up-regulated and 17 microRNAs down-regulated by a greater than 2-fold difference. Target gene and gene ontology (GO) analysis suggests that among the pathways targeted by these microRNAs, there is an enrichment in pathways involved in regulation of apoptosis, nucleotide metabolism, NF-kB signaling, and cytoskeletal organization. We corroborated the up-regulation of MCDC2, an apoptotic regulator. In addition, we have found two virally generated microRNAs, encoded in the c-terminal of the large T-Antigen gene, which provide JCV with innate immune evasion capabilities and help its replication cycle.
S4: Peripheral neuropathies
Introduction: the procedures and utility of nerve biopsies nowadays
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The indications for nerve biopsy (NB) have declined over the last 20 years, due to progress in molecular biology for diagnosing genetic neuropathies (PN) and to the increasing use of skin biopsy. The latter is mainly employed to evaluate small-fiber PN, and it rarely discloses the etiology of a PN.

Usually, NB concerns mostly either the sural or the superficial peroneal nerve, which is combined with removal of specimens from the peroneus brevis muscle. The diagnosis of vasculitic lesions can be readily established on nerve paraffin-embedded fragments, although in some cases, characteristic lesions are only visible on muscle specimens.

Other nerve specimens are fixed in buffered glutaraldehyde and prepared for semi-thin sections, while frozen specimens are prepared for immunofluorescence study. Electron microscopy (EM) is of great value in some cases of “atypical” chronic inflammatory demyelinating polyneuropathies (CIDP), when the clinical and electrophysiological European Federation of Neurological Societies/Peripheral Nerve Society criteria are absent and in PN associated to a monoclonal gammapathy (MG). In these dysimmune neuropathies, treatments according to the mechanisms of lesions of the nerve parenchyma are quite efficient.

With respect to genetic PN, more than 60 genes may be involved; if the most frequent gene mutations have not been detected, detailed pathological analysis of the nerve can orient the search for mutations in specific genes.

ImmunoEM may be very useful in some cases of MG.

So, NB is still of great value provided that it is performed and examined in a laboratory specialized in the field of nerve pathology.

Skin biopsy in neuropathology: indications, techniques and results
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Skin biopsy has become a widely used tool to investigate small caliber sensory in peripheral neuropathies. Different techniques for tissue processing and nerve fiber evaluation have been employed and validated. The quantification of intraepidermal innervation can be useful to detect a pathologic correlate for otherwise unexplained burning pain in the toes or feet, i.e. small fiber neuropathy. Normative values for different body areas have been established. For diagnostic purposes in peripheral neuropathies, one biopsy is performed at the distal leg (10 cm above the lateral malleolus) and a further one at the proximal thigh (20 cm below the iliac spine) in order to gain information on whether the disease is length dependent. Using appropriate immunohistochemical stains, different nerve fiber populations can be investigated in the skin. Quantification of pilomotor and sudomotor nerves gives information on autonomic nervous system involvement in peripheral neuropathy. Recently, the possibility to sample myelinated fibers and mechanoreceptors, particularly in glabrous skin has extended the application of skin biopsy to questions of myelin pathology. In particular, the new area of nodo-paranodopathies may benefit from analysis of Ranvier nodes in dermal nerve fibers. Immunostaining for inflammatory cells may help in the diagnosis of vasculitic neuropathy. Skin biopsy being only minimally invasive also lends itself to serial investigation, such that studies on nerve regeneration in skin have been performed. Quality control measures must be followed at all levels to ensure the optimal use of skin biopsies.

Diagnosis and pathogenesis of neural leprosy
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Leprosy is a highly prevalent infectious peripheral neuropathy in Brazil, India and Africa, which causes physical disabilities and deformities. It mostly affects skin and the peripheral nervous system among other organs. It can compromise exclusively the peripheral nerves configuring the primary neuritic leprosy without cutaneous lesions. The absence of acid fast bacilli in the nerve biopsy specimens makes diagnosis more difficult as the evidences have then, to be based on nonspecific morphological changes of the nerves, on the detection of Mycobacterium leprae DNA in the nerve samples, of serum anti-phenolic glycolipid and the presence of M. leprae antigens in the nerve specimens by immunohistochemistry. A high integration between clinicians, pathologists and laboratory professionals must be achieved in order to increase diagnostic efficiency. In regard of its pathogeny, the participation of metalloproteinas was addressed, showing the capacity of M. leprae to induce the mRNA expression and protein synthesis of MMP9 and MMP2 on Schwann cells line ST88-14 in vitro. The higher expression of MMP9, MMP2 and tissue inhibitor of metalloproteases found in leprosy affected nerves also corroborated the in vitro findings and these elevated expression was associated with the presence of inflammatory infiltrate and fibrosis. Nerve fibrosis is one of the main features of neural leprosy and M. leprae induces transferrerinhibition of Schwann cells to myofibroblasts a property that can be involved in the intense collagen deposit found in leprosy-affected nerves. Excessive expression of a fibrogenic cytokine such as CCL2 was detected in leprosy nerves and could contribute to the end-stage fibrosis.

Pathological findings in peripheral nerve lymphomas
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Introduction: Neurolymphomatosis (NL) is a rare presentation of lymphoma in peripheral nerves.
Objective: To determine the electrophysiological, radiographical, pathological findings and neuropathy pattern in NL.
Methods: Retrospective review of NL patients proven by nerve biopsy.
Results: 48NL patients were identified (28 primary and 20 secondary NL). The most common presentations were asymmetric, painful radiculoplexus neuropathy (37.5%), polyradiculoneuropathy (23%) and multiple or single mononeuropathy (18%). Primary and secondary NL presented similarly with primary NL having longer symptom duration (10 vs. 6 months, p<0.001), single disease episode (96% vs. 35%, p<0.001), disease remission (58% vs. 20%, p=0.056) and lower mortality (25% vs. 67%, p=0.054). 11 of 42 patients had electrophysiology showing demyelination. Nerve biopsies diagnostic of lymphoma were distal cutaneous (sural; 9/11) or proximal targeted fascicular (35/35). MRI was better identifying lymphoma than CT or PET (84% vs. 46% vs. 58%). The lymphomas were B cell (46/48; only two T cell) with diffuse large B cell most common. Teased fibers showed increased demyelination (19/24), axonal degeneration (18/24) and empty nerve strands (9/24). Endoneurial lymphoma was common (43/48) follow by epineurial (29/33). Pathology of primary and secondary NL were similar with secondary NL having more axonal degeneration (31% vs. 7%; p=0.0005).

Conclusion:
1. Peripheral nerve lymphoma (NL) is a multifocal painful neuropathy that causes endoneurial inflammatory demyelination.
2. Primary NL presents as single episodes. Secondary NL presents after remission suggesting that nerve may act as a “safe lymphoma haven” – most chemotherapeutic agents cannot cross the blood nerve barrier.
3. Primary NL is less severe with longer disease duration, less axonal degeneration and higher survival rates.
Nerve biopsy may be very useful in some genetic neuropathies

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At the present time, about 60 genes are known to be linked to Charcot-Marie-Tooth (CMT) diseases: a careful recording of a possible hereditary transmission and of clinical and electrophysiological data may orientate to look at first, for anomalies of the most frequently involved genes, using classical techniques of molecular biology. Actually, PMP22 duplication or P0, Connexin32 and MFN2 mutations will be found in many cases. Then, detailed pathological analysis of the nerve lesions can orientate the search for mutations in specific genes. The various characteristic pathological lesions encountered in CMT diseases according to the mutated gene will be presented and discussed.

If a hereditary sensory neuropathy is suspected, NB is not necessary. Nevertheless, only EM can detect a severe and characteristic involvement of the unmyelinated axons as it is in amyloid neuropathy. Moreover, morphological patterns suggestive of a hereditary neuropathy may be detected in nerve biopsies from patients with seemingly sporadic neuropathy of unknown etiology. Such disorders as amyloidosis, giant axonal neuropathy, polyglucosan body disease and infantile or juvenile neuroaxonal dystrophy are now rarely diagnosed by a nerve biopsy.

The peripheral nervous system is often involved in various lipidoses: Fabry’s disease, metachromatic leukodystrophy, adrenoleukodystrophy, etc. Nowadays, a gene mutation has been identified in many of these disorders, so that molecular biology is now firstly considered as a diagnostic procedure.

The use of new-generation sequencing methods by selecting a great number of gene variants could benefit of combining for each patient not only clinical and electrophysiological data, but also microscopic observations.

LS1: Recent advances in the neuropathology and neurobiology of epilepsies

The first international consensus classification of hippocampal sclerosis

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Hippocampal sclerosis (HS) has long been recognized as most common structural lesion in patients with temporal lobe epilepsy (TLE), and presents with a broad spectrum of electro-clinical, structural and molecular pathology findings. In past decades, various attempts have been made to classify specific neuropathology patterns of hippocampal neuronal cell loss and correlate subtypes with postsurgical outcome. A task force from the Diagnostic Methods Commission of the International League against Epilepsy (ILAE) reviewed previous classification schemes and proposed a system based on semi-quantitative hippocampal cell loss patterns that can be applied in any histopathology laboratory. Inter- and intra observer agreement studies reached consensus to classify three types in anatomically well preserved hippocampal specimens (HS ILAE Type I, II or III; details will be presented). Surgical hippocampus specimens obtained from TLE patients may also show normal content of neurons with reactive gliosis only (no-HS). This first international consensus classification will aid in the characterization of specific clinico-pathological syndromes, explore variability in imaging and electrophysiological findings, and postsurgical seizure control.

Developmental tumours and focal cortical dysplasia: improving management by abandoning simplistic views

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It is debatable whether developmental tumours (DT) and focal cortical dysplasia (FCD) are part of a neuropathological continuum or represent distinct entities. Because surrounding cortical dyslamination is so frequently associated with DT, this debate impacts upon presurgical evaluation and surgical treatment of refractory partial epilepsies due to DT. The new ILAE classification of FCD recently fueled this controversy by creating a category in which cortical dyslamination (i.e. FCD type I) is associated with DT, comprising the ‘new’ category FCD type III. For this to be clinically meaningful, it is important to decide on the epileptogenic relevance of the dysplastic abnormalities (i.e. dyslamination) associated with the DT. More specifically, it is important to determine whether dyslaminated cortex surrounding a DT can generate seizures on its own, or whether it is of low epileptogenic relevance – and seizures are solely dependent on the DT. The problem is compounded by the fact that intermingled with the dyslamination there are often satellites of the tumour and it is unclear whether persisting seizures following lesionectomies are due to remaining ‘dysplasia (i.e. dyslamination)’ or subtotal resection of the tumour (i.e. persistence of satellites).

We will present a tailored approach to epilepsy-related DT, abandoning the simplistic view that all patients should be evaluated with intracranial EEG electrodes, on the rationale that the seizure onset zone is otherwise uncertain. Taking into consideration clinical, scalp EEG and imaging features, we will report the Porto Alegre series of epilepsy-related DT operated under acute ECoG guidance. We will make the point that decades of experience with costly and risky intracranial EEG evaluations do allow more objective, successful approaches.

Prevention of epileptogenesis and neoepileptogenesis in temporal lobe epilepsy: are we getting closer?

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Temporal lobe epilepsy with hippocampal sclerosis is the most common type of intractable partial epilepsy. Surgical removal of mesial temporal structures may lead seizure freedom. However, surgery may fail and a substantial number of patients will continue to be refractory to drug treatment. Therefore, prevention of epileptogenesis after a primary brain insult could be an inventive method to reduce pharmacoresistant epilepsy. Here, we will address the current knowledge on the underlying mechanisms involved in epileptogenesis, and on the potential novel treatments that may lead to prevention of intractable epilepsy. So far, all clinical and the vast majority of experimental studies have fail to truly prevent (cure) epilepsy after a brain injury. Nevertheless, some experimental studies have shown that prophylactic treatment with different compounds may lead to diminished brain damage, reduction of frequency and severity of spontaneous seizures and reduction of behavioral and cognitive deficits. Such preventive approaches have diverse underlying mechanisms of action and include antiepileptic drugs, neuropeptides, immunosuppressant and anti-inflammatory agents, neurotrophic factors, nucleosides and even proconvulsant drugs.

One of the reasons that may explain the relatively low success of such therapeutic approaches is our limited knowledge of the pathophysiology of epileptogenesis after a brain insult such as status epilepticus or traumatic brain injury. The choice of animal model to test these
compound also seems relevant and a rational approach with combination of models that allow screening large number of drugs with more complex and time-consuming models such as kindling or animals with spontaneous seizures should be encouraged.

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Focal cortical dysplasia associated with perinatal ischemic injury
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Cortical dysplasia frequently presents with epilepsy resistant to drug therapy, and when localized becomes a candidate for treatment by surgical resection. The consensus classification of focal cortical dysplasia (FCD) by the ILAE task force in 2011, rightly proposed separation of cortical abnormalities associated with other pathologic lesions into a separate category, and FCD associated with lesions acquired during early life, other than with vascular malformations, has been designated FCD Type IId.

Epilepsy surgery resections for lesions resulting from perinatal ischemic injury range from small cortical and larger lobar resections to hemispherectomies, and the principal abnormalities found in these specimens consist of porencephaly with microgyri and ulegria. The latter two are also seen as isolated lesions. The adjacent cortex may be thin and attenuated with disorganized architecture and absence of laminarization, or represented by nodules of gray matter. The cortex may appear thick and marbled, with aberrant myelinated axons running vertically up between groups of neurons. In some cases, cortical laminarization is maintained, but neurons are reduced in number. Focal microcolumnar arrangement of neurons is often found in cortex adjacent to the main lesion. Hypertrophic neurons are frequently found in the microgyri and the surviving crowns of ulegria and may be found in the better preserved cortex further away.

Recognition of these changes as secondary to the perinatal insult is not difficult in large resections with an appropriate clinical history. It may be more challenging in resections of small lesions in older subjects with neuroimaging “suggestive of cortical dysplasia.”

Anti-angiogenesis and hypoxia in gliomas: what have we learned
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A critical barrier to progress in the treatment of patients with glioblastomas (GBMs) who receive bevacizumab (Avastin®) combined with chemotherapy is the yet unexplained resistance to anti-angiogenic therapy. Although an improvement in quality of life and performance status is the subject of debate, these patients have experienced a prolongation of their progression-free survival, but not overall survival, compared to historical controls, and relief from steroid dependence due to diminished tumor edema. However, most patients eventually relapse and die of their disease, with unprecedented tumor dissemination due to acquired or intrinsic resistance to bevacizumab.

Using clinical specimens, cell culture and animal models of gliomas to investigate these molecular events, we and others found that bevacizumab treatment increased tumor cell invasiveness and expression of hypoxia-regulated molecules, such as the transcription factor HIF-1α as well as the CXCR4 and c-MET receptors at the invasive edges of tumors (Zagzag, 2006, 2008; Esencay, 2010; Lu, 2012). We hypothesize that vascular remodeling triggered by bevacizumab leads to a more hypoxic tumor microenvironment that may promote glioma cell invasion into the normal brain parenchyma, as noted during the development of resistance. Our studies provide novel insight into the hypoxic reprogramming of glioma tumors and their mechanisms of resistance to bevacizumab, and may help in the development of new therapeutic approaches to benefit patients with GBM. In addition to GBM therapy, new applications of bevacizumab to the treatment of neurofibromatosis 2-associated vestibular schwannomas and radiation-induced cerebral necrosis have been reported.

Experience with anti-angiogenic therapy in clinical practice
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Glioblastoma (GB), a highly aggressive malignant primary brain tumor, is among the most vascularized tumors, and relies upon angiogenesis for growth and histological progression. Angiogenesis in GB, like all solid tumors, is mediated primarily by vascular endothelial growth factor (VEGF), which is also an important vascular permeability factor, and is therefore the primary culprit behind vascular cerebral edema, a major cause of morbidity in brain tumor patients. Bevacizumab (BV), an anti-VEGF monoclonal antibody, became the first clinically validated antiangiogenic therapy for recurrent GB. BV demonstrated significant clinical activity in non-comparative phase II studies used as a single agent or in combination with chemotherapy. Clinical activity was mostly based on reduced need for steroids, impressive radiological responses and promising survival rates. BV introduction into clinical practice brought some challenges, like how to evaluate tumor response, and a wide spectrum of toxicities, such as hypertension, thromboembolism, and especially delayed wound healing and bleeding.

Currently, the clinical benefit of adding BV to first-line standard therapy is being discussed with the publication of two phase III clinical trials, showing a lack of overall survival benefit and some contrasting results regarding quality of life, despite an improvement in progression-free survival. Finally, despite some advances, many significant treatment challenges remain. There is an urgent need to validate predictive biomarkers for patient selection and monitoring the efficacy of...
anti-VEGF treatment. In addition, more work is needed to better understand the biology of both tumor response to antiangiogenic therapy and the mechanisms of tumor escape.

WS1: Surgical neuropathology challenges: new and not so new entities

CLIPPERS

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CLIPPERS, a CNS inflammatory disease, was defined by Pittock et al. in 2010 as a steroid-responsive and steroid-dependent relapsing-remitting disease, combining clinical brainstem attacks, characteristic punctate and curvilinear enhancing lesions involving the pons and/or the middle cerebellar peduncles, and the presence of perivascular lymphocytic infiltrates. This mainly T-CD4 infiltrate had a very low specificity. The role of the neuropathologist is to rule out differential diagnoses, especially vasculitis and lymphoma and to suggest the hypothesis of CLIPPERS in a multi-disciplinary approach.

Nevertheless, two reported cases of typical CLIPPERS have evolved into primary CNS lymphoma (PCNSL) and one into primary CNS lymphomatoid granulomatosis (PCNS-LYG). In these three patients, some unusual findings could challenge the diagnosis of CLIPPERS: remnant gadolinium enhancement after steroid therapy, asymmetrical involvement of the brainstem, relapses becoming less sensitive to steroid.

As suggested by Taieb et al., CLIPPERS could sometime be a pre lymphoma state, representing an effective host immune response (i.e. sentinel lesion in PCNSL or PCNS-LYG grade I in LYG) preventing a clonal proliferation of B-cells. When this immune response becomes too weak, B-cell lymphoma or PCNS-LYG grade III could emerge. Pittock et al. suggested another hypothesis: CLIPPERS might be an autoimmune disorder triggered by an unknown perivascular antigen. Then in some rare CLIPPERS patients, chronic antigenic stimulation might lead to malignant transformation of B-cells targeting this antigen.

Finally, CLIPPERS could be a syndrome rather than a single disease, including initial stage of CNS B-cell lymphoma and LYG.

N-methyl-D-aspartate (NMDA) encephalitis

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The current concept of autoimmunity is based on the presumed balance of self-reactivity and processing of “foreign” antigens. Abnormal CNS autoimmunity results from an in equilibrium between genes, molecular mimicry and environmental factors. NMDA-receptor encephalitis was first described in 2007 (Dalmau et al., Ann Neurol) and since then, a whole host of antibodies against synaptic receptor proteins (Casp2, LGI-1, GABA-B, mGluR3, glycine receptor, etc.) have been revealed. The early reports of NMDA receptor encephalitis have shown the involvement of IgG antibodies against the NR1a subunit of the NMDA receptor. Patients with schizophrenia were reported to have IgG antibodies against NR1a/NR2b (Steiner et al., JAMA Psychiatry Jan. 2013). Antibodies to NMDARs have been recently described in diseases that had not previously been thought to have an autoimmune component (CJD and HSV encephalitis). The exact pathogenesis of the neuronal damage is as yet to be clarified and much remains to be done to identify any morphological abnormalities, particularly since biopsy and/or autopsy material to be analyzed is scarce. In sight of this recent plethora of reports, it seems useful to review the diagnostic criteria for anti-NMDAR antibody encephalitis and related morphological observations. The latter partly are our own observations (Barry et al., Br J Psychiatry 2011). The present review shows that GluN1 IgG antibodies are specific for the syndrome of anti-NMDAR encephalitis. GluN1 IgM or IgA antibodies may be associated with the other pathologies (Panzer et al., J Neural Transm 2014).

IgG4 encephalitis

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IgG4-related disease has evolved from originally being recognized as a form of pancreatitis to encompass diseases involving numerous organs that may occur in isolation, in various combinations, and in the absence of autoimmune pancreatitis.

In the CNS, IgG4-related disease has been described affecting the pituitary gland (IgG4-hypophysitis) and the dura/meninges (IgG4-related pachymeningitis). Involvement of the brain parenchyma or spinal cord has not been reported thus far. IgG4-related hypertrophic pachymeningitis may account for a large percentage of cases previously considered as idiopathic hypertrophic pachymeningitis, and should be considered in the differential diagnosis of such conditions including infectious diseases, inflammatory disorders, and malignant lymphoproliferative neoplasms.

A recent review of the literature (PMID: 24733677) has reported a total of 33 cases with histologically confirmed IgG4-related pachymeningitis. The majority of the patients were men in the 6th decade of life and the most clinical presentation were related to mechanical compression of either vascular or nerve structures including headaches, cranial nerve palsies and vision problems.

The characteristic histopathological features of IgG4-related diseases are a rich lymphoplasmacytic infiltration of IgG4-positive plasma cells, storiform fibrosis, and obliterative phlebitis, all features present in the IgG4-related pachymeningitis.

The pathogenesis of the IgG4-related diseases is still not known. Recent data support the concept of an antigen-driven disease, which involves collaboration between CD4+ T cells and somatically hypermutated oligoclonal IgG4+ B cells.

Neuroinflammation: A rose by any other name?

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Over the last decade, the term ‘neuroinflammation’ has become a widely used portmanteau without generally accepted neurobiological, neuropathological, or neuroimaging tissue correlates. This is problematic in diseases, such as Alzheimer’s, Parkinson’s or schizophrenia, where patients have been treated under the broad hypothesis that anti-inflammatory medication should be of benefit. We have asked the question whether an unbiased, data-driven in silico approach may help to clarify the confusion. Specifically, we have examined whether unsupervised clustering of microarray data obtained from cerebral cortex of Alzheimer’s, Parkinson’s and schizophrenia patients would reveal a degree of relatedness between these diseases and recognized inflammatory conditions. Our results provide strong evidence against this hypothesis demonstrating that distinct sets of genes are involved: key characteristics of the inflammatory response are recognizable across different organs at the histophenotypic and the transcriptomic level but are not shared with ‘neuroinflammation’ (1). Similarly, work using a newly created knockout mouse (Banati et al., submitted) indicates that expression of binding sites for a mitochondrial microglial regulator, which is commonly ascribed to activated microglia, can be functionally dissociated from classical microglial activation, calling concepts into question that link activated microglia to non-autoimmune ‘neuroinflammation’. It seems particularly important to determine whether microglial activation in the numerous conditions where it is seen (from sleep deprivation to depression and obesity) relates to synaptic changes and whether the bi-functionality of complement (inflammatory role in inflammation and synaptic role in healthy and
By the 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, primary CNS lymphomas (PCNSL) are rare extranodal non-Hodgkin lymphomas occurring in 15 to 25% of all patients. PCNSL represent about 2.0 percent of all primary CNS tumors (CBTRUS, 2013) and intraocular involvement occurs in or around the central nervous system (CNS). Such happens with Rosai-Dorfman disease (RDD), Erdheim-Chester disease (ECD), and Whipple disease (WD), whose unsuspected diagnoses may be the neuropa-thologist’s prerogative to unravel. Intracranial RDD is a deceptively mimicking meningeal and has histologically resemblance the lymphoplasmacytic type. Emperipoleis (engulfment of intact lymphocytes by histiocytes) is often difficult to find and typical RDD patterns are commonly obliterated by dense dural fibrosis. CD68 and S100 positivity favors RDD, whereas CD1a negativity excludes Langerhans cell histiocytosis (LCH). ECD, another skillful mimic of meningeal, is easily overlooked when the characteristic long bone lesions are clinically silent. ECD diagnosis is based on systemic findings and exclusion of RDD and LCH (ECD histiocytes are CD68-positive but S100-negative and CD1a-negative). CNS WD usually manifests itself as lymphocytic encephalitis with numerous PAS-positive histiocytes, the diagnosis being confirmed by PCR or immunohistochemical demonstration of Tropheryma whipplei. Histioctyes are also a prominent feature of LCH (which shows CD68-negative, S100-positive, CD1a-positive dendritic cells), choroid plexus xanthogranuloma (mostly an incidental autopsy finding), and the extremely rare juvenile xanthogranuloma and histiocytic sarcoma. On the other hand, histiocytes are often encountered in the setting of infection, demyelination, infarction, and various tumor types, where they may facilitate or hinder the diagnosis depending on the observer’s awareness.

Primary CNS lymphomas (PCNSL) are rare extranodal non-Hodgkin lymphomas arising in the CNS and/or in the eye in the absence of obvious systemic lymphoma. PCNSL represent about 2.0 percent of all primary CNS tumors (CBTRUS, 2013) and intraocular involvement occurs in 13 to 25% of all patients. By the 2008 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, the terminology PCNSL is restricted to diffuse large B-cell lymphomas (DLBCL) mostly arising in immunocompetent patients. Lymphomas associated with native or acquired immunodeficiencies, monomorphous (mostly low-grade B-cell marginal zone type), intravascular B-cell lymphomas, and T-cell lymphomas (near less than 5% of the cases) are excluded from this diagnosis and should be considered in their related group. PCNSL arise in the sixth and seventh decades of life and have a slight male prevalence. The tumors are usually deep within the cerebrum often involving the basilar ganglia and periventricular regions. Unlike most primary CNS tumors, they are often multifocal lesions, simulating metastatic tumor. The major clinical diagnosis of PCNSL is DLBCL genetically expressing germinal center-associated markers including MUM-1, BCL-6, and BCL-2. However, recent molecular studies have demonstrated that PCNSL have unique gene expression profiling and different than their systemic counterpart. The diagnosis of PCNSL may be a challenging one in several settings including inadequate sampling of the lesions and, most commonly, effects of corticosteroid treatment prior the brain biopsies. In addition, lymphoid proliferations of uncertain malignancy potential including lymphomatoid granulomatosis may present an extra challenge to the pathologist.
therefore discuss future approaches for the classification of epileptogenic brain tumors (“epileptomases”) in order to promote a comprehensive discussion between neuropathologists, neurooncologists and epileptologists.

Guidelines for the WHO classification of long-term epilepsy-associated brain tumors: is there a current need for revisions?

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Long-term epilepsy-associated tumors (LEATs) account for a fourth of surgical specimens obtained for seizure control and include ganglioglioma (GG), dysembryoplastic neuroepithelial tumor (DNT), pleomorphic xanthoastrocytoma (PXA), composite tumors, pilocytic astrocytoma, low-grade diffuse gliomas, and rarer neoplasms, such as papillary glioneural tumor, isomorphic astrocytoma, angiocentric glioma, cortical ependymoma, meningoangiomatosis, meningioma, and extraventricular neurocytoma. They generally share basic clinicopathologic features, including young age of onset, a chronic clinical presentation, low-grade biology, limited potential for malignant transformation (except for diffuse gliomas and PXA), and a predilection for neocortical and/or mesial temporal lobe involvement. Nevertheless, diagnostic criteria within the current World Health Organization (WHO) scheme are often subjective or vague, leading to low concordance rates among even the most expert neuropathologists. Additionally, non-classic LEATs and low-grade glioneuronal tumors are relatively common, particularly in pediatric patients, further complicating the classification of these challenging cases. As such, this lecture will focus on some of the most common diagnostic controversies, rare histologic variants, recent advances, and potentially useful immunohistochemical and molecular markers. Examples of the latter include the strong association of IDH mutations with adult low-grade diffuse gliomas and the common, but more variable associations of BRF V600E mutations and CD34-positive cells with PXA, GG, and DNT. Future efforts by the International League against Epilepsy (ILAE) and the WHO should focus on reproducible, evidence-based diagnostic criteria in order to create as narrowly defined clinicopathologic and molecular entities as possible, as well as to improve interobserver concordance among neuropathologists.

Molecular diagnostic findings in long-term epilepsy-associated brain tumors: the future for classification of brain tumors

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Brain tumors often manifest with epileptic seizures. However, biopsy specimens of patients that undergo neurosurgical removal of circumscribed foci in order to control chronic recurrent pharmaco-resistant seizures often reveal tumor entities that are rarely observed in general brain tumor series. The spectrum of these ‘long-term epilepsy-associated neoplasms’ comprises highly differentiated glial and glioneuronal tumors that show a benign biological behavior, clinical course and rarely relapse. Several entities are well detectable based on histopathological and immunohistochemical characteristics. A frequent feature of respective neoplasms is given by the co-incidence with dysplastic lesions, which are in close neighborhood to the tumor itself. The recent advent of new molecular markers including genomic alterations beyond the nervous system, and found that both peripheral inflammation and behavioral stress modulate the content of PrP C at the plasma membrane of neutrophils, with consequences upon peroxide-dependent cytotoxicity toward vascular endothelial cells. Our data are consistent with the scaffold hypothesis, which explains the multiple roles of the prion protein in physiology and pathology, and further suggest that PrP C may be relevant for various degenerative/noncommunicable diseases.

The role of exosomal secreted Stress Inducible protein 1 in protection against Aβ oligomers toxicity and ischemia

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Stress-inducible phosphoprotein 1 (STI1) is part of the chaperone machinery, but it also functions as an extracellular ligand for the prion protein (PrP). The physiological relevance of these STI1 activities in vivo is unknown. The absence of STI1 is embryonic lethal by E10.5 with mutant mice showing increased caspase-3 activation, 50% impairment in cellular proliferation, placental disruption and lack of cellular viability. STI1 haploinsufficient mice showed increased vulnerability to ischemic insult. Extracellular STI1 prevented ischemia-mediated neuronal death in a PrP-dependent way. In addition, specific binding of AβOs (Aβ oligomers) to PrP is efficiently inhibited by STI1. Treatment with STI1 prevented AβO-induced synaptic loss and neuronal death in cultured neurons and long-term potentiation inhibition in mouse hippocampal slices. Interestingly, STI1-haploinsufficient neurons were more sensitive to AβO-induced cell death and could be...
rescued by treatment with recombinant STI1. Thus, indicating the essential roles for intracellular and extracellular STI1 in cellular resilience. STI1 is released by astrocytes; however, it lacks a signal peptide and does not follow a classical secretion mechanism. Diverse biochemical and electronic microscopy approaches demonstrated its secretion by exosome-like vesicles. STI1 partially co-localized with Rab5 and Rab7 in endosomal compartments, and a dominant-negative for vacuolar protein sorting 4A (VPS4A), required for formation of multivesicular bodies (MVBs), impaired EV and STI1 release. Flow cytometry and PK digestion demonstrated that STI1 localized to the outer leaflet of EVs, and its association with EVs greatly increased STI1 activity upon PrP-dependent neuronal signaling. Thus, indicating that the interaction between EVs and neuronal surface components enhances STI1-PrP signaling preventing neuronal injury by ischemia and AβO toxicity.

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Prion-like properties for tau
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Filamentous inclusions made of hyperphosphorylated tau are characteristic of numerous human neurodegenerative diseases, including Alzheimer disease (AD), tangle-only dementia (TD), Pick disease (PiD), argyrophilic grain disease (AGD), progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD). We previously demonstrated that the injection of brain extracts from human mutant P301S tau-expressing transgenic mice into the brains of mice transgenic for wild-type human tau (ALZ17) resulted in the assembly of wild-type human tau into filaments and the spreading of tau inclusions from the injection sites to anatomically connected brain regions. This is consistent with an intercellular transfer of tau aggregates. We have then injected brain extracts from humans with pathologically confirmed AD, TD, PiD, AGD, PSP and CBD into the brains of ALZ17 mice. Silver-positive inclusions formed in all cases, with the presence of pathological structures reminiscent of ADG, PSP and CBD following the injection of the corresponding human brain extracts. The assembly and spreading of human tau in the ALZ17 brain was independent of Aβ. Similar inclusions also formed after intracerebral injection of brain homogenates from human tauopathies into non-transgenic mice, and the induced formation of tau aggregates could be propagated between mouse brains. Finally, we have shown that the intraperitoneal injection of brain extracts from symptomatic mice transgenic for human mutant P301S tau into presymptomatic transgenic mice promoted the formation of tau inclusions in brain, albeit less efficiently than following intracerebral injection. Altogether, these findings suggest prion-like properties for tau.

Prion-like features in alpha-synucleinopathies
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Filamentous alpha-synuclein (αS) deposition in nerve cells or glial cells is the defining neuropathological feature of Parkinson’s disease, dementia with Lewy bodies (DLB), and multiple-system atrophy (MSA). In these so-called alpha-synucleinopathies, αS is accumulated in a hyperphosphorylated form with beta-sheet-rich, fibrillar structure. During the course of a study of cytotoxicity of αS and tau fibrils, we found that these abnormal fibrils can enter cells and act as seeds for aggregation. I thought that these intracellular abnormal proteins propagate from cell to cell and this spreading can explain the disease progression. Then, we injected synthetic αS fibrils into brains of wild-type mice and found that these mice developed abundant Lewy body/Lewy neurite-like pathology. Immunoblot analysis clearly demonstrated that endogenous mouse αS started to accumulate 1–3 months after inoculation, while injected human αS fibrils degraded in a week. Furthermore, inoculations of Sarkosyl-insoluble αS from DLB and MSA patients induced similar αS pathology. Inoculation of insoluble αS accumulated in wild-type mice into other wild-type mice also induced αS pathology. These results demonstrate that αS fibrils have prion-like properties and αS pathology spreads throughout the brain by this prion-like feature. These cellular and mouse models should be useful for evaluating disease-modifying therapy.

Propagation of TDP-43
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Neurodegenerative diseases are increasingly recognized as sharing a unifying pathological principle, i.e. the accumulation of characteristic protein aggregates in neurons and glial cells that are recognized as the hallmarks or diagnostic lesions of these disorders. Converging lines of evidence indicate that disease progression could be driven by a molecular triad of template-directed protein misfolding, seeded aggregation, and cell-to-cell transmission of non-prion neurodegenerative disease proteins [2]. The major component in ubiquitin-positive neuronal inclusions in sporadic amyotrophic lateral sclerosis (ALS) and a large subset of frontotemporal lobar degeneration (FTLD) is the phosphorylated 43-kDa TAR DNA-binding protein (TDP-43). TDP-43 is a highly conserved and widely expressed RNA-binding protein that is a member of the heterogeneous nuclear ribonucleoprotein family of proteins. The defining histopathology of ALS and FTLD-TDP is the presence of TDP-43 aggregates in select neurons and glial cells of the central nervous system [4]. We recently proposed four stages of TDP-43 pathology in ALS that suggest a spreading of TDP-43 directed outwardly from the motor neocortex, spinal cord and brainstem, whereas later stages are characterized by frontal, parietal and anteromedial temporal lobar involvement [1]. This sequence of involvement (and the neuro-axonal loss linked to cellular accumulation of TDP-43) has recently been corroborated in vivo using fiber-tracking magnetic resonance imaging [3]. The frontal dissemination of TDP-43 pathology could explain why up to 50% of ALS patients develop cognitive deficits related mainly to executive function. We review recent evidence on the dissemination of TDP-43 from cell culture and mouse model experiments as well as from human tissue studies.

W4: Updates in muscle disorders

Congenital myopathies
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Presentation is usually in infancy or early childhood; although late adult onset or clinical heterogeneity among affected members family may also occur. Congenital hypotonia, muscle weakness, facial muscle involvement, ophthalmoplegia, difficulties feeding and respiratory failure may be prominent. CK is low or normal and course may remain stable.

Type 1 predominance/type 1 hypotrophy is typical as are characteristic structural changes, although members of the same family may have only type 1 predominance. Diagnosis may not be conclusive in early biopsies. Inheritance can be autosomal dominant (AD), X-linked, autosomal recessive (AR) or resulting from dominant de novo mutations.

Most frequent entities: Nemaline myopathies, rods visible using Gomori’s trichrome and EM, of variable clinical severity and age of onset, at least eight genes appear to be involved. NEB mutations, mostly de novo mutations, represent almost half of severe congenital cases.
Core myopathies, with central or eccentric oxidative defects, Z line disruption and absence of mitochondria in the cores, may show severe neonatal presentation or milder forms. AD or AR related to RYR1 gene.

CNM has been linked to at least four genes, XR-MTM1, DNM2, BIN1 and RYR1, with severe to mild presentation. Morphology findings may orient genetic studies. Congenital fibre type disproportion CFTD shares genes with most other congenital myopathies. Subsequent biopsies may reveal structural changes. Cases still remain with no genetic identification.

Update on mitochondrial diseases
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Mitochondrial myopathies are caused by defective oxidative phosphorylation and may be caused by mitochondrial DNA (mtDNA) mutations, which can be primary and maternally inherited or secondary to nuclear DNA mutations resulting in reduced mitochondrial DNA copy number or multiple mtDNA deletions. Mitochondrial myopathies may also be caused by nuclear gene mutations that affect the processing of mitochondrial tRNAs or translation of mtDNA encoded polypeptides. In addition, an increasing number of nuclear genes associated with respiratory chain subunits, assembly proteins or other factors of importance for the oxidative phosphorylation have been identified. Clues to correct genetic diagnosis can be obtained by combined morphological, clinical and biochemical investigations. Mitochondrial disorders may rarely present as pure myopathies. Diseases caused by primary mtDNA mutations often present with mitochondrial myopathy with cytochrome c oxidase deficient muscle fibers but important exceptions occur. Mitochondrial myopathy of infancy is sometimes reversible and genetic analysis can be performed to identify such cases. Nuclear DNA mutations with secondary mtDNA alterations such as deletion or multiple deletions usually present with morphological changes in muscle but there are important exceptions. Nuclear DNA mutations causing oxidative phosphorylation disorders present in many cases without evidence of mitochondrial myopathy but there are exceptions e.g. mutations in the iron-sulphur cluster scaffold protein. Defective phosphatidylcholine biosynthesis causes a type of congenital muscular dystrophy with mega-mitochondria with structural abnormalities that is not a primary oxidative phosphorylation disorder. In addition, some muscle diseases including various metabolic diseases and age related myopathies may demonstrate mitochondrial changes, which look similar to those present in primary oxidative phosphorylation disorders.

The genetic approach to muscle diseases
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The neuromuscular disorders are a heterogeneous group of genetic diseases, causing a progressive loss of the motor ability. In the last decade, mutations in several genes have been identified, resulting in the deficiency or loss of function of numerous important proteins. Complementary biochemical and immunohistological analyses have identified these proteins in several compartments of the muscle fiber, including the sarcolemma, extracellular matrix, the sarcomere, muscle cytosol and the nucleus.

Different approaches can be used for the diagnostic, characterization of the diseases, and for the genetic counseling of the family. Both molecular analysis of DNA of candidate genes, and the analysis of the defective protein can be done, according to the clinical hypothesis. Protein analysis can be used for the differential diagnosis and to direct the search for gene mutations, mainly because there is genetic heterogeneity, and most of the known genes are very large and present wide variability of mutations causing diseases. Protein analysis is also of utmost importance for the elucidation of the physiopathology of each genetic disorder involved. Genotype–phenotype correlation through the analysis of the effect of different mutations on protein expression and on phenotypic variability contributes to the understanding of gene function.

Currently, new methods for wide genome screening are becoming more accessible and able to identify a great variety of mutations and polymorphisms in patients and their families. It is still not possible to evaluate whether this information will help improving diagnosis and genotype/phenotype associations or if it will make the diagnostic process more confusing and complex. However, it will be certainly a giant leap in the genetic analysis of this large group of muscle diseases. Supported by FAPESP-CEPID, CNPq-INCT, FINEP, CAPES-COFECUB.
Prion diseases in Latin America

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Creutzfeldt-Jakob (CJD) and other prion diseases have been reported in several Latin American countries. Following the emergence of variant CJD (vCJD) in 1996, enhanced surveillance was implemented in Europe and in some Latin American countries, where prion diseases became reportable disorders. CJD notification became mandatory though not autopsied. Report of cases to referral centers must include clinical data, EEG, MRI and CSF for the detection of protein 14.3.3, requirements similar to those established by WHO and Euro-CJD. Based on provided data, cases are classified as possible, probable or definite.

Confirmed histopathologic diagnosis is not required for cases to be classified as probable. Diagnosis of definite CJD requires neuropathological confirmation and/or positive PrPsc/WB although there may be protease sensitive cases. Definite cases are classified following international consensus criteria based on PrPsc/WB subtype and codon 129 polymorphism of the prion protein gene, histotyping may also be used. Updated decontamination needs to apply and genetic studies made available. Latin American reporting rates vary significantly given the territory's vast extent. Population clusters in large urban centers show levels comparable to those of smaller, high population density countries in Europe. Some countries conduct surveillance for CJD or other prion diseases while others implement monitoring regulations: Argentina's incidence in 2013 was 0.82 cases/million (16% genetic out of a total of 368 definite and probable cases from 1997–2013, 14% CJD E200K mostly Chilean origin, one octarepeat insertion and 4 GSS PrP102L); Brazil reported 201 definite and probable cases for the last 10 years (7.4% genetic cases are mostly PrP202K, 2 GSS PrP102L, 3 PrP105H, 2 FFI and 1 CJD); Chile reported incidence approximately 3.6 cases/million (35% genetic PrP202K); Mexico reported 50–60 cases in the past 15 years (genetic cases associated to PrP202K); incidence in Uruguay is 0.7 cases/million (familial cases associated with PrP1114 and PrP202K); Colombia (Ministry of Health, mainly to exclude vCJD); Paraguay and Panamá. No vCJD has been reported. Countries lacking CJD surveillance: Bolivia; Costa Rica; Cuba; Ecuador; Peru; Venezuela. No information obtained for Dominican Republic, El Salvador; Honduras, Guatemala or Nicaragua. Improved surveillance and greater health authority involvement are needed to avoid underreporting.

Pathophysiology of disturbances in cerebral blood flow in dementia

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Cerebral perfusion is reduced in most forms of dementia, including Alzheimer’s disease (AD) and vascular dementia (VaD). This is likely to be partly physiological, reflecting decline in metabolic demand, and partly pathological, potentially caused by structural disease of the cerebral vasculature (atherosclerosis, arteriolosclerosis and cerebral amyloid angiopathy), functional abnormalities of cerebral vascular contractility and permeability, and extracranial cardiovascular disease (including cardiac dysrhythmias, valvular disease, carotid stenosis and orthostatic hypotension).

To distinguish pathological from pathological hypoperfusion, we measured several ischaemia-sensitive proteins in cerebral grey and white matter. These included vascular endothelial growth factor (VEGF; the synthesis of which is strongly upregulated by hypoxia-inducible factors, the major sensors of cellular oxygenation) and myelin proteins differentially susceptible to ischaemia.

Our investigations revealed pathological hypoperfusion of cerebral cortex in AD and of white matter in VaD, with resulting upregulation of VEGF and selective loss of myelin-associated glycoprotein, a myelin protein particularly sensitive to ischaemia. In AD, hypoperfusion was not significantly associated with structural vascular pathology but was directly related to the severity of AD, as measured by Braak tangle stage and Aβ42 load. We identified upregulation of endothelin-converting enzyme-mediated production of the vasoconstrictor endothelin-1 as a likely contributor to pathological hypoperfusion in both AD and VaD, and specifically in AD, an increase in activity of angiotensin-converting enzyme (which cleaves angiotensin II to produce the vasoconstrictor angiotensin II).

Our findings may help to explain the disparity between the distribution of cerebral atrophy and that of reduction in blood flow and glucose utilisation in early AD, and to illuminate some aspects of the relationship between cerebral hypoperfusion and cognitive decline. The identification of particular biochemical pathways that contribute to pathological hypoperfusion in dementia also suggests possible pharmacological approaches to ameliorating the associated brain damage.
Scheinker diseases (GSS) are difficult to transmit. Finally, prion disease transmissibility is thought to be helped by the structural similarity between the prion protein (PrP) of the donor and that of the host. We have probed the transmission of poorly transmissible prion diseases such as sFI, and variably protease sensitive prionopathy (VPSPr), a sporadic novel prion disease with features similar to GSS, using two sets of Tg mice as hosts: (1) Tg mice expressing normal human cellular PrP (PrP\(^{C}\)) \([\text{Tg(HuPrPC)}]\) and (2) Tg mice expressing human PrP\(^{\beta}\) but free of the sugar moiety \([\text{Tg(HuPrPGlycKO)}]\).

While sFI and VPSPr were transmitted to Tg(HuPrPC) only after very long incubation periods and showed minimal pathology, inoculations to Tg(HuPrPGlycKO) caused illness in less than half the incubation period with very severe pathology characterized by marked neuronal loss, granulomatous-like reaction and massive deposition of scrapie PrP.

These findings suggest that (1) the distinction of transmissible and non-transmissible prion diseases is in principle unfounded; (2) structural similarity between PrP seed and substrate does not necessarily provide the most favorable conditions for transmission; (3) glycosylation may reduce PrP\(^{\beta}\) convertibility and PrP\(^{\beta}\) toxicity in prion diseases. Supported by NIH Award AG14359, CDC UR8/CCCU515004 and Charles Britton Fund.

**Phenotypic heterogeneity in a Brazilian family with a mutation in codon 102 of the prion protein gene**

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About 15% of the prion diseases are caused by different mutations in the prion protein gene (PRNP). P102L mutation is usually associated with Gerstmann–Straussler–Scheinker (GSS) phenotype. **Objective:** To describe the phenotypic heterogeneity in a Brazilian family with P102L mutation.

**Methods:** Patient 1 was seen in 2002 with an atypical dementia. We tracked all data from other affected members of the family.

**Results:** Patient 1: woman, age at onset: 27 years old. Memory and attention deficits; progression to severe dementia in 2 years; gait instability and language disturbances started 5 years later. MRI: brain atrophy, normal DWI, CSF 14.3:3; negative. EEG: non-specific changes. PRNP: 102L; 129M; 102P; 129V. Death: 15 years after onset. Neuropathology: PrPsc immunopositive plaques in the molecular layer of the cerebellum. Minimal spongiosis.

Patient 2 (uncle of no. 1): age at onset: 53 years old. Rapidly progressive dementia with myoclonus. MRI: frontal atrophy. EEG: non-specific changes. Brain biopsy: spongiform encephalopathy. Death: 8 months after onset. PRNP: not available.

Patient 3 (sister of no. 2): age at onset: 54 years old. Dizziness, cognitive decline, cerebellar ataxia and blindness. MRI: brain atrophy. Death: 4 years after onset. PRNP: 102L; 129M; 102P; 129V.

Patient 4 (daughter of no. 3): age at onset: 36 years old. Progressive cerebellar ataxia, spasticity and feet numbness. Mild executive function deficit after 2 years. Severe dementia 7 years after onset. MRI: cerebellar atrophy. PRNP: 102L; 129M; 102P; 129V. Death: 10 years after onset. Neuropathology: neuronal loss, spongiosis and gliosis. PrPsc immunopositive in synapses and in multicentric plaques.

Patient 5 (mother of patient no. 1): age at onset 66 years old. Paresthesias in lower limbs and social withdrawn, followed by cerebellar ataxia and cognitive decline. MRI: non-specific changes. PRNP: 102L; 129M; 102P; 129V. Death: 4 years after onset. Neuropathology: neuronal loss, gliosis, spongiosis and PrPsc immunopositive in synapses and in multicentric plaques.

**Conclusions:** Prion disease associated with P102L mutation presented with high clinical and pathological heterogeneity in this Brazilian family.

**Approaches to therapy in prion diseases**

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Despite the rarity of human prion diseases, significant efforts have been made toward the development of therapeutic strategies. Several compounds have been found to inhibit PrP\(^{\beta}\) formation or enhance its clearance in in vitro models and to prolong survival in experimental animals. However, toxicity, inability to cross the blood–brain barrier or both have left only a few drugs that could be used for clinical evaluation in humans. They include flupirtine, a centrally acting analgesic with neuroprotective activity, the antimalarial quinacrine, pentosan polysulfate (PPS), and the antibiotic doxycycline. Double-blind, placebo-controlled trials in Creutzfeldt–Jakob disease showed that flupirtine, quinacrine and doxycycline have not significant effects on survival. PPS was given to a small number of patients by continuous intraventricular infusion since it does not cross the blood–brain barrier. Although invasive, this treatment seemed to prolong survival in vCJD, whereas there is no evidence of efficacy in other prion diseases.

Among future promising approaches to prion disease treatment is PrP-targeted immunotherapy, modeling strategies presently used for Alzheimer’s disease. Passive immunization with antibodies to PrP\(^{\beta}\) and active mucosal vaccination can protect rodents against prion infection from peripheral source. Humanized versions of effective antibodies identified in in vivo models could be available in the near future for clinical trials in CJD and other prion disorders.

**Variant CJD: dead or dormant?**

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Variant CJD (vCJD) was identified in the UK in 1996; 177 cases have now occurred in the UK, with 52 cases in 11 other countries. vCJD results from infection with the bovine spongiform encephalopathy (BSE) agent. Exposure to BSE though contaminated meat products is likely to have been widespread in 1980–1990s in the UK, when 2–3 million infected cattle may have entered the human food chain. It has therefore been questioned why the total number of vCJD cases in UK is not higher and is now in decline. Potential factors include a protective effect of the “species barrier” between cattle and humans, the infectious dose required to achieve transmission, age-related susceptibility, and genetic factors: all patients with pathologically confirmed vCJD are homozygous for methionine at codon 129 in the prion protein gene. A series of retrospective tissue-based prevalence studies were undertaken on surgically resected appendix and tonsil samples from otherwise healthy individuals in the UK who were likely to have had been exposed to BSE. The disease-associated form of the prion protein accumulates in lymphoid tissues in vCJD and has been identified in appendix tissues surgically removed from vCJD patients before the disease onset. The latest and largest of these prevalence studies (Gill et al. BMJ 2013; 347: f5575) found 163/2,441 appendixes to be positive, indicating a prevalence of asymptomatic vCJD infection of 1:2000 in the UK. Given the possible risks of secondary transmission of vCJD by blood transfusion and surgical instruments, continued surveillance for vCJD is required in the UK.

**Understanding the unique biology of prions by in vitro prion replication**

Soto C

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Transmissible spongiform encephalopathies are a group of fatal and infectious neurodegenerative disorders affecting humans and various brain structures.
species of mammals. These diseases are associated to an unconventional form, named prion, that seems to be composed exclusively by a misfolded form of the prion protein (PrPSc), which propagates by transmitting its misfolding to the normal prion protein (PrPC). The lack of a procedure to cultivate prions in the laboratory has been a major limitation to the study of the unorthodox nature of this infectious agent and the molecular mechanism by which PrPC is converted into the infectious PrPSc isoform. Protein misfolding cyclic amplification (PMCA) is a simple, fast and efficient methodology to mimic prion replication in the test tube. PMCA consists of incubating materials containing minute amounts of infectious prions with an excess of PrPC and boosting the conversion by cycles of sonication to fragment the converting units, leading to accelerated prion replication. PMCA is able to detect the equivalent of one single molecule of infectious PrPSc and propagate prions that maintain high infectivity, strain properties and species specificity. Since its invention 13 years ago, PMCA has helped to answer fundamental questions of this intriguing infectious agent and has broad applications in research areas including the food industry, blood bank safety and human and veterinary disease diagnosis.

S6: Tumors: a multidisciplinary approach
The role of contemporary neuroimaging in guiding patient management in neuro-oncology
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Patient management in neuro-oncology is dependent on tumor type, tumor grade and tumor extension.
In addition to conventional magnetic resonance (MR) imaging techniques, advanced techniques including diffusion-weighted imaging (DWI), proton magnetic resonance spectroscopy (H-MRS), perfusion-weighted imaging (PWI) and dynamic contrast enhanced (DCE)/permeability studies can provide information beyond mere anatomy and may help estimate tumor type, tumor grade and to assess tumor extension.
1. Determination of tumor type
High-grade gliomas, lymphomas and metastasis may look very similar on contrast enhanced MR images. Accurate preoperative differentiation between these tumors is important for appropriate treatment. The information obtained from advanced MR imaging techniques may help indicate a specific diagnosis.
2. Determination of tumor grade
Differentiation between high-grade and low-grade tumors is important for therapeutic planning. The contrast-enhancement pattern of a tumor on conventional MR imaging is not always reliable enough to obtain precise information about tumor angiogenesis because tumoral enhancement is mainly due to disruption of the blood-brain barrier (BBB) rather than the tumoral vascular proliferation itself. Advanced MR imaging techniques may better estimate tumor grade than conventional MR imaging studies.
3. Assessment of tumor extension
Determination of tumor extension is essential for therapeutic planning (surgery as well as radiation therapy). H-MRS as well as PWI may show tumor infiltration beyond the enhancing tumor nodule demonstrated on the conventional contrast enhanced images. In conclusion, modern advanced MR imaging techniques such as DWI, H-MRS, PWI and permeability may provide valuable information to neuro-oncologists and neurosurgeons for therapeutic planning that is not available with conventional MRI.

Update on the morphologic and molecular features of pediatric brain tumors
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Morphologic diversity has always been a distinguishing feature of pediatric brain tumors. More recently, high throughput molecular profiling has greatly enhanced our understanding of the molecular basis of cancer in general and pediatric brain tumors in specific. Broad categories that have been further refined by recent scientific advances include pediatric gliomas, embryonal tumors and ependymomas. Among gliomas, those of low grade represent the most frequent primary brain tumors in children. Pathologically, they correspond to World Health Organization (WHO) grades I or II and include pilocytic astrocytoma, pilomyxoid astrocytoma variant, angiocentric glioma, subependymal giant cell astrocytoma, diffuse astrocytoma, oligodendroglioma, and pleomorphic xanthoastrocytoma. Activating BRAF mutations/rearrangements and increased MAPK pathway signaling are universal features of circumscribed gliomas, such as pilocytic astrocytoma. Although all glioma subtypes may develop in children and adults, clinical and molecular differences have become evident particularly in the diffuse glioma group. Alterations in FGFR1, MYB and MYB1 are frequent events in pediatric low grade diffuse gliomas while mutations in chromatin remodeling proteins (e.g. ATRX) or histone proteins (H3.3) characterize diffuse intrinsic pontine gliomas and subsets of pediatric glioblastomas. The embryonal neoplasm category is represented predominantly by medulloblastoma, which has been classified into four main molecular subtypes: WNT, SHH, group 3 and group 4. Similar molecularly based classification schemes have been proposed for primitive neuroectodermal tumors and ependymomas. The consequence has been a move toward more objective classification schemes that incorporate histopathologic and molecular information, and that have increasing prognostic and therapeutic relevance in neuropathology and neurooncology practice.
Current treatment options for brain tumor patients
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While primary malignant brain tumors account for only 2% of all adult cancers, these neoplasms cause a disproportionate rate of cancer-related disability and death. The 5-year survival rates for brain tumors are the third lowest among all types of cancer. Malignant gliomas are the most common primary brain tumors. They are associated with the shortest survival time explained by their early recurrence due to their deep invasion of the normal brain, which makes them practically impossible to remove completely. Invasive anaplastic gliomas are almost invariably fatal, recurring close to the resection margin in almost all cases.

A dramatic increase in knowledge regarding the molecular biology of brain tumors has been established over the past few years. In particular, recent new avenues regarding the role of stem cells and microRNAs along with further understanding of the importance of angiogenesis, immunotherapy and explanations for the resistance of the tumors to chemotherapeutic agents and radiation therapy has been developed.

As an example of the application of the molecular biology to the clinic, it can be mentioned the long-term results of two landmark trials, RTOG 9402 and EORTC 26961 for anaplastic oligodendrogial tumors that indicate that patients whose tumors harbor a 1p/19q co-deletion benefit particularly from the addition of procarbazine/lomustine (CCNU)/vincristine (PCV) chemotherapy to radiation therapy (RT). The median survival of patients with co-deleted tumors was 14.7 years compared with 7.3 years of patients who received RT alone. In contrast, no such benefit was observed for patients with tumors lacking 1p/19q co-deletion.

S7: Forensic neuropathology
Chronic traumatic encephalopathy: update on an emerging disease
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The association of chronic head trauma and progressive neurodegeneration with neurofibrillary tangles, also known as chronic traumatic encephalopathy (CTE), has been known in boxers (“dementia pugilistica”) for decades, but nearly 10 years ago, we reported the first case of chronic traumatic encephalopathy (CTE), has been known in boxers (“dementia pugilistica”) for decades, but nearly 10 years ago, we reported the first case of CTE in an American National League Football player bringing this hitherto obscure condition to national and international attention. Despite a demand for retraction of this paper by members of the NFL Mild Traumatic Brain Injury committee, the paper stood and the second case of CTE in an NFL player was reported shortly afterwards. Since that time, over 100 cases of CTE in the modern era have been evaluated from American football, football, ice hockey, entertainment wrestlers, battered spouses, and American military veterans as well as boxers. At this time, any condition in which a person is subjected to repeated head trauma should be considered as a potential risk factor for the development of this condition. Currently, the epidemiology of CTE is unclear, and the incidence and prevalence of this disorder are unknown. Clinical histories of head trauma and participation in contact sports should be documented for all patients presenting with cognitive or motor symptoms suggestive of a neurodegenerative process. We are at the early stages of understanding this condition: unraveling the clinical-pathological correlations and the pathophysiology will likely take decades and can only be accomplished by cooperation between neuropathologists, neurologists, major sports organizations and military medical facilities.

Neuropathological findings in a soccer player
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Recent studies corroborate and expand previous reports associating sport-related traumatic brain injury (TBI) to a higher risk of progressing neurodegenerative changes. In fact, the neuropathological features underlying sport-related TBI seems to constitute an independent pathological entity characterized mainly by deposits of phospho-tau proteins with contribution of other abnormal protein inclusions. Soccer is an extremely popular sport worldwide. Well-executed headings are considered one of the most beautiful parts of a soccer game. Headings require critical skill and exhaustive practice that can lead to chronic TBI. However, very few reports approached the underlying neuropathological changes in soccer players. Here we report an 82-year-old former professional soccer player who developed dementia during his last decade of life. His cognitive decline was well-characterized for several years and resembled Alzheimer’s dementia. Neuropathological assessment disclosed moderate Alzheimer’s disease stages. In addition, chronic traumatic encephalopathy changes characterized by superficial cortical phospho-tau deposition in glia and neurons alike, more concentrated in the gyral depths were identified especially in frontotemporal areas. Finally, hippocampal sclerosis and abnormal TDP-43 deposition in temporal and ventral frontal cortical and subcortical structures complete the report. The features of this case resemble those described for other sport-related TBI and further investigation are necessary to clarify to which extend soccer practice can lead to chronic TBI.

Traumatic brain injury caused by gunshot wounds
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According to the mechanism of injury, head trauma can be divided in two types: those caused by FAP (firearm projectile) and those not caused by FAP (mainly by vehicle accidents, falls and assaults). In most caused by FAP, head gunshot wounds are the leading cause of brain injury directly involved with death. The severity of brain injuries basically depends on the velocity and shape of the projectile. The most commonly used are low velocity projectiles, but war weapons (rifle) with high-velocity projectiles are also used. The main objective of this presentation is to show different characteristics of gunshot wounds and mechanisms of brain injury produced by projectiles of low and high velocity in forensic autopsies.

Chronic traumatic encephalopathy: the military setting
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Mild traumatic brain injury (TBI) includes concussion, subconcussion and most exposures to explosive blast from improvised explosive devices. Mild TBI is the most common TBI affecting military personnel and may produce persistent, long-term, effects. Military TBI tend to be less stereotyped and less predictable than sports-related TBI, and the chronic effects of military TBI are often heterogeneous. A single TBI can produce gray and white matter atrophy, precipitate or accelerate age-related neurodegeneration, including Alzheimer’s disease, and repetitive mild TBIs can provoke the development of a tauopathy, chronic traumatic encephalopathy (CTE). CTE has been reported most often in athletes, although early changes of CTE were found in five young veterans of the Iraq and Afghanistan conflict who were exposed to explosive blast, four of whom were also diagnosed with posttrau-
mastic stress disorder. Advanced CTE has also been found in veterans who experienced repetitive neuroruma while in service, although in general, the pathology tends to be more variable than that found in individuals with exposure to trauma exclusively from sports. Clinically, CTE is associated with behavioral changes, executive dysfunction, memory loss and cognitive impairments that begin insidiously and progress slowly over decades. Pathologically, CTE produces atrophy of the frontal and temporal lobes, thalamus and hypothal- 
mus, septal abnormalities, and abnormal deposits of hyperphospho-
rylated tau as neurofibrillary tangles and disordered neurites through- 
out the brain. The incidence and prevalence of chronic trau- 
matism encephalopathy and post-traumatic neurodegeneration after 
military TBI and the genetic and other environmental risk factors 
critical to their development are currently unknown.

Traumatic brain injury in children
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Traumatic brain injury (TBI) in childhood occurs most frequently 
during the first 5 years of life and the later teenage years. While in 
the second group of patients, injuries are most likely acquired during 
sporting activities and due to motorized vehicle accidents (MVAs), 
non-accidental TBI corresponds to a large proportion of cases seen in 
infants with national incidences reported as high as 24.6 per 100,000 
children. In infants, due to the relative elasticity of the skull, fractures 
are infrequently seen while intracranial hemorrhages, usually subdural 
hemorrhages, are common. Increased intracranial pressure due to 
hemorrhages with compression of the brain, cerebral contusions fol-
lowing impact of the brain with the internal surface of the skull and 
dura, and shearing injuries within the cerebral white matter due to 
rapid acceleration/deceleration of the head are common findings in 
pathological examination of these cases. In addition, intraocular 
hemorrhages including retinal and peri-optic nerve hemorrhages have 
been reported to occur in about 80% of children who are known to 
suffer non-accidental TBI. Their presence on autopsy should raise the 
suspicion of non-accidental trauma.

Red neuron, do we really know it?
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There are some central nervous system changes related to anoxic-
ischemic injury that could be detected under the microscope, and 
probably the most important is the presence of the so called “red 
neuron” in certain areas of the brain. Most of the studies describing 
the geographical appearance – or timing – of that cellular change 
were performed in animal models of anoxia and/or ischemia; however, 
human studies and reports are sometimes required to clarify certain 
issues in the judiciary field, especially in the area of “dating” lesions 
or express the minutes required – of an injury – to produce a micros-
copic change.

S8: Develage
The Develage concept: linking pathways common to 
developing and aging brain
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The molecular and cellular mechanisms that are critical in stages of 
brain development to reach a normal structural organization with 
appropriate networks are progressively being elucidated. Recent 

Neuroinflammation in neurodegenerative diseases 
with abnormal protein aggregates: why current 
therapies do not work?
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Institute of Neuropathology, Bellvitge University Hospital- 
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Neuroinflammation is common in the majority of neurodegenerative 
diseases with abnormal protein aggregates in old age. This is mainly 
manifested by activation of microglia and by abnormal brain expres-
sion of cytokines and mediators of the immune response. However, 
parallel studies in several neurodegenerative diseases and related 
animal models show that the inflammatory responses differ from one 
disease to another, with the progression of a given disease and among 
different brain regions in a particular stage of the disease. Moreover, 
advanced stages of diseases show a plethora of mixed responses inclu-
ding up-regulation of pro- and anti-inflammatory cytokines and 
factors that modulate degeneration and regeneration pathways. Neu-
roinflammation in neurodegenerative diseases differs from neuroin-
flammation related to aging and it is not a mere acceleration of it. 
Finally, neuroinflammatory responses in animal models, although 
similar, are not exactly the same as those seen in human diseases, thus 
compromising a direct translation of neuroinflammatory responses in 
animal models, mainly murine transgenic models, to human patients. 
Together, these observations may explain, at least in part, why current 
anti-inflammatory therapies do not work in established stages of 
human neurodegenerative disease although they are beneficial in 
rodent models. These observations also point to the need to identify 
tailored therapies geared to modulating (activating or inhibiting) spe-
cific molecules involved in neuroinflammation at the early stages of 
the neurodegenerative processes, ideally before the appearance of cli-
nical symptoms.

Neurogenesis in human brain during development 
and aging: when does youth end and old age begin?
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We report the development of hippocampal formation in human 
fetuses from 8 weeks of gestation (GW) to adult stage. Multiple immu-
nohistochemical labelings were performed to study the progenitors 
using Ki67 associated with nestin, SOX2, PAX6 and TBR2, and neu-
ronal markers using NeuN, CUX1, SATB2, CTIP2, TBR1, NeuroD1, 
doublecortin, CaBP and Calretinin.
The gradient of neurogenesis started in the entorhinal cortex, extending toward the ammonic plate. The proliferation of Pax6+ or Sox2+ progenitors was already observed at 9 GW in the ventricular zone (VZ). Intermediate-stage progenitors labeled by Tbr2 were present at 11 GW in the subventricular zone. The density of progenitors progressively decreased around 20GW. Ammonic pyramidal neurons appeared during the early fetal stage, labeled with Tbr1, CTIP2 and Cux1. They were produced directly from founding and indirectly from intermediate-stage progenitors. The lamination of the pyramidal layer follows an inside-out gradient, producing a three-layered ammonic plate, whereas the entorhinal cortex recapitulated neocortical neurogenesis.

The dentate gyrus (DG) developed from the VZ next to the hem from nine GW and contained SOX2/Kis1+ progenitors. The secondary dentate matrix adjacent to the dentate VZ appeared around 11 GW, composed of Pax6+, Sox2+ and Tbr2+ proliferating progenitors and migratory cells. It extended toward the DG following a subpial migration route and established the proliferative tertiary dentate matrix in the hilus. Granule cells were mainly labeled by Cux1. Progenitors persisted in the subgranular zone in young children, but significantly declined in adults.

The research leading to these results was performed in the frame of DEVELAGE project “Pathways common to brain development and ageing: defining strategies for preventive therapy and diagnostics” (HEALTH-F2-2011-278486) and has received funding from the European Community’s 7th Framework Programme (FP7/2007-2013).

Neurodegeneration in the developmental period: a continuing role for the morphologist in the genetic era
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Utilizing recent examples from the author’s clinical practice, this presentation considers the impact of genetic degenerative disease on the developing human brain as evidenced by both the intermix of morphologic features and their precocious onset. Three widely differing clinical scenarios—a malformation syndrome with neuro-axonal dystrophy in sibling fetuses, a rare familial dystonia, and connatal spinal muscular atrophy SMA1A—are discussed. While the underlying genetic aberrations range from the completely unknown, through the recently determined, to the established, in these instances the neuropathological analysis brings new insights into the pathogenesis of these disorders. With the current explosion in genetic data, and the rapid advances in neuro-imaging, the opportunities for neuropathological investigation are dwindling. And yet the need for morphologic data in guiding genetic screening, formulating pathogenetic hypothesis, and aiding clinical management and prognosis remains urgent.

Long-distance plasticity: extensive rewiring in the brain of human subjects born without the corpus callosum
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It is well known that humans born without the corpus callosum lack the disconnection syndrome classicly described in callosotomized patients. However, this paradox discovered by Nobel laureate Roger Sperry in 1968, remained unsolved since then. We tackled the hypothesis that alternative neural pathways could explain this intriguing clinical presentation, and investigated patients with callosal dysgenesis using structural and functional neuroimaging, and neuropsychological assessments. Two anomalous white matter tracts were identified by deterministic and probabilistic tractography, and proved functional by resting state functional neuroimaging and neuropsychological evidence, playing a functional role in preserved interhemispheric transfer of complex tactile information such as object recognition. These compensatory pathways connect the homotopic posterior parietal cortical areas (Brodmann areas 39 and surroundings) via the posterior and anterior commissures. Other aberrant tracts were found connecting hom- and heterotopic cortical regions, of unknown function. We propose that anomalous brain circuitry in callosal dysgenesis is determined by long-distance plasticity, a phenomenon occurring in the developing brain after pathological interference. As possibly occurs in other developmental brain anomalies, these pathologic changes somehow divert growing axons away from their normal pathways, with partial compensatory effects or deleterious consequences to the patients. Long-distance plasticity, therefore, should be conceived as a major property of the developing human brain subjected to pathological circumstances, causing extensive rewiring either capable of compensating loss of function, or of generating symptoms. The findings show that, together with the search for a human brain connectome, it will be important to investigate the disconnection of subjects with congenital brain diseases.

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LS3: Neurodegeneration: Alzheimer’s disease
Distribution and significance of Abeta: the issue of preclinical (prodromal AD) and resilience to Alzheimer neuropathologic change (pathological aging)
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Alzheimer’s disease (AD) is neuropathologically characterised by the presence of amyloid-β (Aβ) in plaques, hyperphosphorylated tau (HPT) in neurofibrillary tangles (NFT) and neuropil threads (NT) and neuritic plaques that contain both Aβ and HPT. However, these lesions are only associated with clinical dementia if their respective severity and topographical distribution passes a critical threshold, while they are present to a lesser degree in clinically non-demented individuals. The latter has been referred to as pre-clinical AD and may neuropathologically be diagnosed as low/medium AD neuropathologic change. On the other hand, subjects that show NFTs but lack Aβ are currently classified as having no AD neuropathologic change.

Granulovacuolar degeneration (GVD) and cerebral amyloid angiopathy (CAA) are associated with AD. In a large autopsy cohort (n=766) we found that no AD controls showed GVD and CAA in 28.6% and 5.4%, respectively, suggesting that those cases might biologically indeed represent pre-clinical AD. Soluble/dispersible Aβ aggregates and pyroglutamylated Aβ were significantly elevated in AD compared to pre-clinical AD, suggesting that specific Aβ species might play a crucial role in the conversion from pre-clinical AD to AD. Further studies are warranted to elucidate if some individuals with pre-clinical AD do not develop AD and if this resilience to AD is partly due to a lack of specific Aβ species.

Tau pathology in aging and AD: beyond neurofibrillary tangles (grains, astrocytes, etc.)
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In 1975, a protein essential for microtubule assembly was isolated and designated tau. In 1986, it was shown that neurofibrillary tangles in Alzheimer’s disease (AD) contain tau protein. Since then, brain tissue obtained at autopsy or during surgery has been frequently assessed for occurrence of pathologically altered tau protein. Today, we know that in certain pathological conditions, altered tau protein tends to accumulate in nerve cells and glia. There are several primary tauopathies.
such as Pick’s disease, progressive supranuclear palsy, corticobasal degeneration among others but the commonest disorder displaying tau pathology is AD. Since the beginning of 1990, we know that in AD, the pathological altered tau seems to first appear in the transentorhinal cortex and then spread to neocortex, i.e. neurodegeneration seem to spread in certain manner from one neuroanatomical region to another. Noteworthy, AD-related lesions are seen in unimpaired aged subjects and the primary difference between an aged subject and a subjects suffering from dementia seems to be the level of spread. In line with the spread of AD pathology in humans, in 2009, it was shown that in transgenic animals, altered human tau spread from one site to neighboring brain region and thus a prion-like propagation pattern was suggested. Finally in 2011, it was published that pathological tau is seen in lower brainstem prior to transentorhinal cortex already at the age of 10 years. Thus, hyperphosphorylation of tau seems to be an early phenomenon that affects most of us in more or less deleterious manner.

Relation between tau and Abeta stages: where does AD begin and end? Are amyloid and tau pathologic processes linked?
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For the last 20 years, 1003 post mortem cases of Escourrolle Neuro-pathology Laboratory have been assessed according to Braak stages (BS) for neurofibrillary tangles by only four observers. More recently, the amyloid deposits were also assessed by Thal phases (TP) in 162 cases among which 48 cases with Lewy bodies and 26 Creutzfeld–Jakob disease cases, recruited by the National Surveillance Center. We analyzed retrospectively BS and TP. Their combination was significantly different from a random distribution. When displayed in a table where the BS and the TP were respectively lines and rows, most of the cases were located on a diagonal, indicating a parallel increase in BS and TP, from BS I, TP 0 to BS V and VI, TP 5. The least affected cases had only tau pathology (BS I, TP 0) – suggesting a precession on amyloid. Seventy one percent of the cases fall on the diagonal; 15% were above (too many AB deposits) and 13 below (too many tau aggregates). Loss of brain weight was significant only for the high BS and TP. There was no difference in the prevalence distribution in cases with Lewy bodies or with CJD.

Neuropathologic variants of AD and the problem of mixed dementia (Abeta, tau, α-synuclein and TDP-43)
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Alzheimer’s disease (AD) pathology is defined by Aβ deposits in senile plaques and tau-positive neurofibrillary pathology. Lesion distribution is stereotopic, leading to staging schemes (Braak for tau and Thal for Aβ). A subset of AD does not fit Braak staging, and many AD cases have mixed pathology, most notably concurrent α-synuclein or TDP-43 pathology. Tau pathology in AD disseminates from medial temporal lobe to association and finally primary cortices. The most severe tau pathology is in medial temporal lobe structures, including hippocampus and amygdala. About 10% of AD have severe cortical tau pathology with minimal involvement of hippocampus (“hippocampal-sparing AD”) and about 10% have severe tau pathology in medial temporal lobe with minimal cortical involvement (“limbic predominant AD”). These AD variants differ from typical AD in demographic features, clinical presentations, and genetic risk profile. It is increasingly recognized that more than 50% of AD have α-synuclein pathology (Lewy bodies and Lewy neurites) and that the distribution of this pathology is different from that seen in Parkinson’s disease and dementia with Lewy bodies. Most often affected are olfactory bulb and amygdala. The clinical expression of olfactory- and amygdala-predominant α-synuclein pathology in AD remains poorly defined. Abnormal TDP-43 is found in more than 50% of AD cases, with or without hippocampal sclerosis. These cases usually have a more severe clinical and pathologic phenotype, but most present with an Alzheimer-like clinical syndrome, not frontotemporal dementia. While AD has been studied extensively for decades, neuropathologic studies continue to highlight molecular and anatomical heterogeneity.

LS4: LEICA digital – teleneuropathology
Teleneuropathology: over 10 years experience at the University of Pittsburgh
Wiley C
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Telepathology is an attractive solution for providing neuropathological intraoperative expertise to geographically diverse hospitals. The University of Pittsburgh Medical Center is a 20-hospital system in Southwest Pennsylvania in which the pathology department has adopted a subspecialty centers-of-excellence method of managing cases. The Division of Neuropathology is physically located at one hospital but provides neuropathological expertise to the entire system. We describe our experience in providing remote intraoperative neuropathological consultations over a 12-year period, from 2002 to 2014. Several approaches were discussed, with emphasis on the current system and the evolution of imaging technology. Diagnostic outcomes are compared and the results show that telepathology can be used to diagnose challenging tumors. Current technology is capable of facilitating teleneuropathological intraoperative diagnoses in a timely manner, with accuracy rates comparable to conventional methods. However, the practice of providing these remote consultations requires a sophisticated and technologically advanced environment along with substantial planning, communication, and training of both pathologists and pathology assistants. After a decade of intra-institutional teleneuropathology, we expanded our practice to cross state lines and communicate between geographically and financially separate medical centers. The result was an effective means of distributing neuropathological expertise while at the same time preserving a professional center of excellence. While technical and legal barriers were surmounted, expected and unexpected issues related to communication required commitment on the part of multiple individuals with diverse expertise and responsibilities. Lessons learned from this successful venture can be used to facilitate future efforts in this ever-growing practical vehicle for distributing pathology subspecialty expertise.

The experience of the University of São Paulo, Brazil
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Autopsies played and, until present days, play a significant role for the advancement in neuropathology. In a variety of pathological conditions, the definition of the precise anatomical locations of the brain alterations, the access of tissues belonging to parts of the brain that are not routinely obtained in the clinical setting, allowing the determination of robust structural, biochemical and molecular information, is certainly of high value for the understanding of neurological diseases. Our department coordinates a large autopsy service, which performs about 14,000 autopsies of natural deaths per year. We recently incorporated to the service a 16-channel CT scan, a 7-Tesla magnetic resonance machine, as well as a system to conduct post-mortem angiographies, though the BIAS project (Brazilian Image + Autopsy Study). One of the main applications of this system is the study of cerebral diseases, coupled to a large brain bank designed to evaluated dementias and psychiatric disorders. In our presentation, we will discuss some of the basic approaches that we employ and present some applications of the combination of advanced imaging
Conventional teleneuropathology: the new aspects of virtual microscopy, and the legal implications in international telediagnostic

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Telemedicine links medical practitioners and remote hospitals to specialized facilities by means of telecommunication in a very fast electronic manner. Teleneuropathology in particular is the work of a neuropathologist over a distance who is viewing digitized images on a video monitor rather than directly through a light microscope. There are two basic imaging systems, namely static and dynamic imaging. Static imaging teleneuropathology can be performed online by transmitting interactive still images for diagnostic purpose, or offline in a store and forward modus.

Technologically improved systems of dynamic imaging teleneuropathology can again be divided in dynamic-robotic and dynamic-virtual approaches. Dynamic-robotic teleneuropathology allows for investigating interactive live images in real time by teledriving a roboticized microscope at the remote local site of the teleneuropathologist, whereas the telemicroscope creating the images is situated at the surgery department of the hospital. In dynamic-virtual teleneuropathology, interactive live images are generated by scanning of histological glass slides, transmitted in real time, and investigated by using a virtual microscopy program allowing for active control by the teleneuropathologist.

Legal aspects of teleneuropathology comprise confidentiality and security, access authentication, tracing of access and transactions, case authentication including encryption, and technical security in an appropriate level such as transmission of encrypted patient’s data separate from transmission of data of digitized histology slides, automatic disconnection if not active, firewalls, and VPN-connections in the Internet. In international teleneuropathology, complicated legal questions may arise, e.g. on responsibility and legal liability, commercial observers from abroad, reimbursement, but also on malpractice and scientific fraud avoidance.

W5: Basic research in tumors: stem cells in tumors

Distinct behavior of glioma stem/progenitor cells upon BMP4 or Noggin treatment

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Recently, glioma stem cells (GSC) have been isolated from patient biopsies and characterized as a small subset of stem-like tumor cells capable of initiating and sustaining tumor growth when grafted into mice. Significantly, GSC lines from different tumors exhibit divergent gene expression signatures and distinct differentiation behaviors.

In the present study, we successfully established six glioma stem/progenitor cell (GSPC) lines derived from high-grade gliomas, which exhibit stem cell properties and initiate malignant brain tumors following xenotransplantation. All established cell lines presented the same genomic alterations as parental tumors and were positive for stem cell markers (Nestin, Vimentin, CD44 and Sox2). Despite similarities between GSPC and neural stem cells, we hypothesized that there may be differences between their differentiation potentials. To address this issue, we exposed these cell lines to several differentiation conditions that mainly include recombinant BMP4 addition and growth factors withdrawal. After analyzing the expression of various lineage markers and assessing changes in cell morphology, we determined that these GSPC lines possess a varying degree of multipotency. Moreover, we observed that addition of BMP4 markedly reduced the proliferation rate of all tested cell lines. Finally, we determined the expression levels of different components involved in BMP-signaling and found that GSPC lines show characteristic expression patterns.

Microglia as a new treatment target in malignant glioma

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The central problem of malignant gliomas, the most common primary brain tumors, is the diffuse infiltration of their neoplastic cells. The fact that it is a difficult problem is evident from the dire survival statistics, which have not changed very much after decades of intensive research. And it is unlikely that much progress can be made in future as long as the core problem is not addressed. One could, therefore, say that most glioma research has been and is not very useful because it ignores this particular challenge. Thus, there is a need for new ideas and experimental approaches.

It is our goal to develop a new treatment for glioblastoma using genetically enhanced microglia precursors obtained from bone marrow. As a first step, we are establishing a background-free imaging model, which allows us to visualize the infiltrating syngeneic tumor in a new global knockout mouse that lacks a molecule expressed by the tumor. MicroPET imaging is being employed and this novel technical approach has successfully established that the tumor can be detected in vivo with a uniquely high signal/noise ratio. We are also working on the establishment of a reverse version of this model where only glioma cells lack this molecule, which will allow us to visualize the infiltration of the experimental glioblastoma by macrophages/microglia.

The long-term vision of this work is to make the glioma-associated macrophages/microglia carry a payload that can be activated for therapeutic purposes using radiation allowing monitoring in real time. First results will be presented.

*Corrections added on 3 February 2015, after first online publication. The complete title and full list of authors are now provided in the abstract.

Molecular pathology of schwannomas

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Schwannomas can be divided clinically as sporadic and syndromic. Syndromic schwannomas include schwannomas that develop in association with the neurofibromatoses (NF2, schwannomatosis) or in association with Carney’s syndrome. Syndromic schwannomas are characterized by unique molecular and histological/immunohistochemical phenotypes that may aid in their recognition.

In addition, schwannomas may be divided according to site of origin and growth pattern (vestibular, peripheral, spinal, cutaneous) or histological subtypes (conventional, cellular, melanotic, plexiform, cellular, epithelioid). Some histological subtypes may pose diagnostic challenges (such as cellular schwannomas or hybrid lesions). Recent studies of schwannomas have discovered involvement of novel genes, in addition to NF2 gene, in schwannoma genesis (LZTR1, SMARCB1 and BRAF). Incorporation of molecular analyses and immunohistochemical stains into the pathological study of schwannomas may provide insight into underlying associated syndromes, tumor biology and in the future, may guide therapy.
Anti-CD47 antibody synergizes with prazosin to promote phagocytosis and eradicate glioblastoma

Kahn SA, Zhang M, Gholamin S, Mitra S, Chneiweiss H, Cheshier S, Weissman I
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GBM patients are currently treated with a standard protocol that consists of surgical removal of the tumor mass followed by radiation therapy together with temozolomide chemotherapy. This protocol is, nevertheless, ineffective since there is recurrence of the tumor followed by an inevitable fatal outcome.

CD47 is a cell surface protein that imparts a signal to the macrophages to “don’t eat” the CD47-positive cells, thus protecting these cells from macrophage-mediated phagocytosis. We demonstrate that CD47 is expressed on all analyzed brain tumors and that the blockade of CD47-induced protection with monoclonal antibodies against CD47 (anti-CD47) stimulates potent tumor phagocytosis in vitro and in vivo. Prazosin, an α-adrenergic receptor antagonist, is a clinically approved drug, and has been adopted in daily clinical practice to treat hypertension. We have found that Prazosin selectively eliminates GBM stem cells in vitro and in vivo, by PKCδ-dependent caspase-3 activation, without altering neural stem cells survival, and increases “eat” signals, such as phosphatidylserine and calreticulin, in GBM stem cells plasma membrane.

This combination therapy induces macrophage-mediated phagocytosis of GBM stem cells in vitro and eliminates the tumor in 50% of the mice, avoiding the recurrence of the tumor in the long term. This therapy can, therefore, be more effective than the current standard protocol for GBM patients.

Brachyury: a novel tumor suppressor gene in gliomas

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Prognosis of malignant glioma patients remains dismal, mainly due to the diffuse infiltrative nature of tumor cells. Epithelial to mesenchymal transition (EMT) is a critical process in tumor invasion in multiple cancer types, including gliomas. Brachyury, also known as T, is a gene that codes for a highly conserved transcription factor of T-box family. Despite its function on embryo mesoderm differentiation, Brachyury role in adult tissues is less understood. Recent studies reported the oncogenic role of Brachyury in several solid tumors, due to its upregulation and promotion of EMT. In gliomas, Brachyury role is unknown.

Herein, we showed for the first time that Brachyury is highly expressed in healthy brain tissues and is lost or reduced in gliomas. We found that reduction of Brachyury is associated with poor patient survival, and with several in vitro aggressive features, including increased proliferation, invasion, EMT processes and stemness profile. Our results demonstrate that Brachyury behave as a suppressor gene in gliomas, at variance with previous described oncogenic effect in other types of solid tumors.

WS6: Brain Tumors in Children – Case Presentations

Challenging cases of small blue cell tumors in children
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Small blue cell tumors of the central nervous system in children often fall in the category of embryonal tumors. According to the 2007 WHO classification of brain tumors, there are three types of embryonal tumors: medulloblastoma, atypical teratoid/rhabdoid tumor (AT/RT), and primitive neuroectodermal tumor (PNET). PNET tumors are further subdivided into neuroblastoma, ganglieneuroblastoma, medulloepithelioma, ependymoblastoma, and PNET-not otherwise specified (NOS). The most prevalent embryonal tumor of the CNS is medulloblastoma. Diagnosis can be challenging by morphologic criteria alone. Recent advances in molecular pathology have allowed us to better classify some of these embryonal tumors. However, not all small blue cell tumors fall into this relatively straight forward category.

Here I will be presenting two cases of CNS tumors with small round blue cell morphology with unexpected diagnosis. The first case is that of an infant with a cerebellar tumor initially diagnosed as a medulloblastoma, who after complete gross total resection and appropriate therapy had tumor recurrence. The second case is that of a teenage child with a large middle fossa mass, composed of small round blue cells and vacuolated cytoplasm. The importance of meticulous histologic examination in combination with immunohistochemistry and molecular studies will be emphasized to elucidate the appropriate diagnosis in these small round blue cell tumors.

W7: Recent advances in bacterial, fungal and parasitic neuroinfection

Brain dysfunction in sepsis
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Systemic infection is often revealed by or associated with brain dysfunction, which is characterized by an acute global alteration of cognitive functions (mostly attention and thinking) and by an altered level of consciousness (ranging from hyper-alertness to lethargy) thus constituting a clinical continuum with delirium. Its pathophysiology involves an ischemic and a neuro-inflammatory processes. Neuro-inflammation is secondary to endothelial activation, blood-brain barrier (BBB) dysfunction with the passage of neurotoxic mediators and astrocyte dysfunction and microglial activation. Microcirculatory dysfunction – also secondary to endothelia activation – contributes to the ischemic process, along with systemic circulatory impairment. This brain dysfunction is associated with increased mortality and morbidity but also long-term cognitive disability that can affect up to 70% of survivors at one year. This supports a relationship between neuro-inflammation and neurodegeneration, although cognitive results might result from the ischemic process. Occurrence of encephalopathy in a septic patient implies a systematic diagnostic approach of all potential factors, additionally to sepsis that contributes to the brain dysfunction, including drugs toxicity or withdrawal, liver/renal failure, metabolic disturbances, alcohol withdrawal or even a vitamin deficiency encephalopathy. Neurological examination is indispensable for appropriately undertaking specific investigations, such as electrophysiological testing, neuroimaging or cerebrospinal fluid analysis (that is indisputably required when meningitis is suspected). Alcohol withdrawal delirium or Wernicke’s encephalopathy are the main differential diagnosis. Currently, SIBD treatment consists mainly on controlling sepsis. Various drugs acting on sepsis-induced BBB dysfunction, brain oxidative stress and inflammation have been tested in septic animals but not yet in patients.
Early neuropathological changes during sepsis
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Septic shock is a major cause of mortality in intensive care units (Annane et al., Lancet 2005). We found in 40% of survivors a neurological achievement at the shutdown of sedation under the shape of a confusion entering within the framework of a septic encephalopathy or delirium, the latter being associated with higher mortality (Eidelman et al., JAMA 1996). Similarly, septic shock is an independent risk factor for progression to dementia disorders compared with other pathologies of resuscitation (Hiwashina, Ely et al., JAMA 2010). It was suggested that alterations of the blood-brain barrier (BBB), resulting from microglial and endothelial activation (van Gool WA et al., Lancet 2010), could explain these anomalies by increasing the passage of inflammatory mediators and neurotoxic substances into the central nervous system (CNS) (Iacobone et al., Crit Care Med 2009). However, there are currently only limited studies on the pathophysiology of sepsis associated encephalopathy. We decided to set up an animal model and to monitor early neuropathological changes during sepsis. For studying microglial cell morphological changes, we developed a 3D automated tissue imaging system by confocal microscopy coupled to mathematical modelisation for studying microglial cells. Microglial cell are extremely dynamic and their morphology is representative of their function. Due to a complex « dendritic like » shape, precise morphological cell parameters are not easy to determine. The knock-in mouse model CX3CR1 GFP, is extremely useful to visualise microglial cells within the brain parenchyma showing precisely all cell processes in details. In this model, with our approach, we observed a very rapid activation of microglial cells that pre-exist to BBB dysfunction.

Cognitive dysfunction after cerebral malaria
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Memory is vulnerable to a variety of pathological process including infectious diseases. Cognitive impairments are frequently detected in children surviving cerebral malaria (CM). We evaluated mechanisms of cognitive impairment after experimental CM induced by Plasmodium berghei Anka (PbA) infection. We found an intense inflammatory response in the mouse brain that coincides with the clinical signs of CM and is characterized by increased levels of TNF-α, IL-1, MCP-1 and IL-12. Intravital microscopy of the brain microcirculation revealed leukocytes and PRBC adherent to microvessels. We detected an increase in oxidative stress in the brains of CM animals with enhanced malondialdehyde and decreased amounts of free thiols. We have also observed activation of microglia detected by increased immunoreactivity for Iba-1 on hippocampal CA1, dentate gyrus and cortex regions of PbA-infected mice. Infected mice also presented positive fluorescence to Fluoro Jade B (neuronal degeneration marker) on day 7 post-infection. Also, mRNA levels for nNOS and NMDAr1 and levels of cleaved caspase-3 are enhanced during experimental CM while synaptophysin is reduced on infected mice. Mice rescued from the infection by chloroquine treatment were tested for cognitive function on day 16 post-infection. Animals recovered from infection presented cognitive decline for both aversive and contextual memories and lower levels of PSD-95 and mature BDNF. We suggest that CM triggers excitotoxic mechanisms that could be responsible for neuronal loss and cognitive decline in animals recovering from CM. Understanding of the mechanisms that are associated with cognitive decline following CM will be an important step towards identification of therapeutic targets and effective therapies.

Neurocysticercosis: clinicopathological correlation
Pittella J
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Neurocysticercosis (NCC) is the central nervous system infection with the encysted larva of Taenia solium, the pork tapeworm. NCC may be asymptomatic or cause a variety of clinical manifestations. The clinical presentation of NCC results from the combination of factors related to the parasite and to the human host: (1) type of cysticercus: C. cellulosae x C. racemosus; (2) presence of viable or degenerated cysts; (3) location of the cysticercus; (4) number and size of cysts; (5) host immune-inflammatory response. Seizures are the most common and may be the sole clinical manifestation of NCC. They are more frequent after the beginning of the parasite degeneration, associated with the immune-inflammatory response. Recurrent NCC-associated seizures have been reported associated with hippocampal sclerosis. Intracranial hypertension is the second most frequent clinical manifestation of NCC and is explained by two mechanisms: (1) direct blockade of the cerebrospinal fluid circulation by leptomeningitis, and ventricular cysts; (2) mass effect produced by large cysts or a large number of small cysticerci. Focal neurological signs are less common and are usually related to (1) large cysts compressing brain structures or the spinal cord; (2) thickening of the leptomeninges at the base of the brain entrapping cranial nerves; (3) stroke syndromes caused by cerebrovascular lesions. Cognitive impairment is more frequent than previously suspected and range from poor performance on neuropsychological testing to severe dementia. Cognitive deficits in NCC seem to be determined to the synergistic interaction among number, localization, and presence of viable or degenerated cysts, and the immune-inflammatory response.

W8: An update on pituitary adenomas and sellar region masses
New insights on familial pituitary tumor syndromes and their implications in sporadic tumors
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The vast majority of pituitary adenomas are sporadic tumors. However, approximately 5% of the pituitary adenomas occur with familial aggregation [multiple endocrine neoplasia type 1 (MEN1), multiple endocrine neoplasia type 4 (MEN4), Carney complex (CNC) and familial isolated pituitary adenoma (FIPA)]. In addition, it was recently described that mutations in the succinate dehydrogenase (SDH) genes can also predispose to pituitary adenomas. FIPA, an autosomal dominant disease with a variable penetrance, is defined as the occurrence of at least two cases of pituitary adenomas in a family that does not exhibit any other features of MEN1, MEN4 or CNC. Inactivating germ line mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene are identified in 20% of patients with FIPA syndrome and in 50% of homogeneous somatotroph FIPA families. AIP-positive somatotropinomas often have an aggressive clinical behavior and show a poor response to somatostatin analogues. Interestingly, AIP protein expression is also reduced in approximately 50% of sporadic somatotropinomas, especially in more aggressive cases, but no somatic AIP mutation has been found. Recently, we have demonstrated that the microRNA miR-34a is overexpressed in sporadic somatotropinomas with low AIP protein levels in the absence of mutations in this gene. Functional studies confirmed that miR-34a down-regulates AIP expression. Altogether, these data suggest the involvement of AIP and miR-34a in the pathogenesis of a subset of sporadic somatotropinomas.
TTF-1 expressing sellar region tumors: a new category of sellar tumors
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Although much less frequent than the main tumor of the sellar region – the pituitary adenomas – pituicytomas, granular cell tumors and spindle cell oncocytomas are important entities in the differential diagnosis of sellar masses. All three tumors are low-grade, non-endocrine neoplasms of the sella that can clinically mimic non-functioning pituitary adenomas. Pathologically, these tumors may be a challenge to diagnose due to their overlapping morphological and immunohistochemical features. Recent data have revealed that the floor of the diencephalon is an area under the influence of the thyroid transcription factor-1 (TTF-1), a transcription factor known to be critical for the development of lungs and thyroid, and now discovered to play a role in the induction of the infundibular anlage during development of the posterior pituitary and infundibulum. The modified glia of the posterior pituitary and infundibulum – the pituicyte – is embryologically derived from the floor of the diencephalon and likewise expresses TTF-1 during embryological development and adult stages. Similar to normal, non-tumorous pituicytes, these three tumors – pituicytomas, spindle cell oncocytomas, and granular cell tumors – show diffuse nuclear expression for TTF-1 providing some insight into the possible common histogenesis of these rare sellar tumors. Additionally, diffuse TTF-1 expression in these tumors provides a diagnostic marker to distinguish them from other entities including adenomas, schwannomas and meningiomas.

What is new in inflammatory pituitary lesions
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Hypophysitis is an uncommon inflammatory disorder of the pituitary gland, which may involve adenohypophysis, neurohypophysis, infundibulum, or combined sites. It is becoming increasingly recognized that medications such as immunomodulatory drugs (ipilimumab) can cause hypophysitis. In addition, isolated cases of IgG4-related hypophysitis have also been described. Primary lymphocytic hypophysitis is no longer a disease simply of pregnant and postpartum women, but rather occurs in both men and postmenopausal women, where it clearly has a different etiology. Recent studies have demonstrated that xanthomatous/xanthogranulomatous hypophysitis shows differing demographics from lymphocytic and granulomatous hypophysitis, with younger age at diagnosis and longer duration of symptoms. This type of hypophysitis, may be related, at least in some instances, to leakage/rupture of Rathke cleft cysts.

Somatostatin and dopamine receptor expression in pituitary adenomas: implications for clinical management
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Pituitary adenomas can be classified as non-functioning or functioning, which comprises prolactinomas, somatotropinomas, corticotropinomas and tiretropinomas. Transsphenoidal surgery is the first-line treatment in most cases, except for prolactinomas. Somatostatin analogs (SA) and dopamine agonists (DA) are the main medical treatment used in patients with pituitary adenomas. The SA act through interaction with the somatostatin receptors, which are divided in five subtypes (SSTR1 to 5). Similarly, DA interacts with dopamine receptors, which are also divided in five subtypes (DR1 to 5). The expression of these receptors can be evaluated by several techniques, mainly PCR and immunohistochemistry. The most commonly used SAs are octreotide and lanreotide, which interact mainly with SSTR2 and to a lesser degree to SSTR5. On the other hand, the main DA available (cabergoline and quinagolide) interact basically with DR2. Both SSTR and DR are found in pituitary adenoma cells in variable amounts, depending on pituitary adenoma subtypes and individual characteristics. It has been shown that the efficacy of SA and DA is dependent on the degree of the receptors expression. Recently, pasireotide, which binds to SSTR1, 2, 3 and 5, has been approved for the treatment of patients with corticotropinomas. Moreover, several SA, with different patterns of interaction with the SSTR, are under investigation. As well as chimeric molecules, which can bind to both SSTR and DR. So, the study of the SSTR and DR expression in pituitary adenomas can guide the choice of drug to be used, leading to a more individualized and cost-effective treatment.
Background: Brain-related tumors are generally classified as gliomas and can be graded as low- or high-grade gliomas. Lower grade gliomas (LGGs) (WHO grades II, III) are histologically and clinically distinct from glioblastoma (GBM) (WHO grade IV). Currently, genome-wide DNA methylation levels have been used to classify a diverse pool of tumors through the TCGA project and recently we have evaluated the DNA methylation profiles of both low- and high-grade gliomas.

Methods: We performed a comprehensive genome-wide DNA methylation of 509 LGGs and 396 GBMs, integrated the analysis with available genomic data and clinical outcomes to identify biologically robust LGG/GBM subtypes.

Results: Unsupervised clustering of genome-wide data derived from DNA methylation platforms uncovered concordant classification of six robust groups each with distinct molecular and clinical patterns. The first three subtypes (LGm1-3) were distinct in their epigenetic profile as compared with non-tumor brain samples and G-CIMP cases as previously identified in the GBM cohort were among this cohort. LGm4-6 on the other hand was predominantly of the high-grade brain tumors and contained 18% of the LGG cohort. GBM-like subset within the GBM cohort was epigenetically similar to the GBM set. One subset among the LGm4-6 was identified as normal-like by its epigenetic profile and was highly enriched for BRAF-fusion events. They were also much younger and had the best survival. LGm4-6 were 100% IDHwt, had high leukocyte infiltration and relatively poorer survival compared to LGm1-3. LGm1-3 had low leukocyte infiltration and was generally classified as long-term survival. This group was also younger at the time of diagnosis compared with LGm4-6 and had IDH mutant somatic alterations.

Conclusions: Epigenetic profiles of the brain tumor cohort identified distinct subtypes with pronounced clinical alterations. We identified sets of samples which may have been misclassified by conventional histological profiling. Brain tumor samples that are epigenetically similar to non-brain tumor samples are probably of a different tumor biology and likely similar to Pilocytic Astrocytoma Grade I. The findings define distinct molecular and clinical features for brain related tumors that may provide greater insight into both the tumor biology and clinically relevant biomarkers.

The human T-cell lymphotropic virus type 1 (HTLV-1) is a retrovirus that infects about 20 million people worldwide. Originally described in association with a hematological malignancy named adult T-cell leukemia/lymphoma HTLV-1 can also cause a chronic slowly progressive myopathy named “HTLV-1-associated myelopathy/tropical spastic paraparesis” (HAM/TSP). HAM/TSP emerges as the tip of the iceberg among numerous other neurological clinical syndromes caused by the virus such as inflammatory myopathies, polynuropathies, ALS-like syndromes, dysautonomia, etc. HAM/TSP manifests as a spastic paraparesis with neurogenic bladder, and minor sensory signs. Pathologically, HAM/TSP is initially characterized by perivascular lymphocytic cuffing and mild parenchymal mononuclear infiltrates mainly in the thoracic spinal cord which is followed by gliosis and scarring. The neuropathogenesis of HTLV-1 is still poorly understood. The therapy of TSP/HAM remains basically symptomatic.

Oli-go? Or no-go?

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Pathologists have long been bedeviled by the distinctions between astrocytoma, oligodendroglioma, and oligoastrocytoma, driven by the perception that the prognosis improves as one proceeds along this spectrum. Oligodendrogliomas were put on a firm foundation when the now classic 1p/19qcodeletion and responsible unbalanced translocation were recognized. Astrocytomas, initially defined in part by mutations in p53 and gain of chromosome 7, were later additionally noted in most cases to have mutations in ATRX. As well the last is associated with the ALT mechanism of telomere maintenance, whereas telomerase assumes this role in oligodendrogliomas. In terms of histological features and clinical outcome, oligoastrocytomas have been, overall, intermediate between astrocytoma and oligodendroglioma, with this position being used to justify the entity. Oligoastrocytomas are also intermediate in terms of molecular features, but only as a group. Individual lesions almost always have either a molecular profile of astrocytoma or one of oligodendroglioma, not both. Most are “astrocytic,” with abnormalities in p53 and ATRX. On the basis of current knowledge, there appears to be little basis to consider oligoastrocytoma as a well-defined clinicopathological entity.
The rise, shine and future of GBM-O
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Prior to the 2007 World Health Organization (WHO) classification of tumors of the central nervous system (CNS), diffuse gliomas histopatologically showing both a substantial astrocytic and oligodendroglial component and features of high-grade malignancy including necrosis were generally designated as anaplastic (WHO grade III) oligoastrocytomas (AOAs). Nevertheless, there existed great inconsistencies among neuropathologists in the diagnoses of these tumors and even within the WHO scheme published in 2000, the glioblastoma with oligodendroglial component (GBM-O) was illustrated and briefly discussed. After studies showed that the prognosis for patients with an AOA with necrosis was more similar to that for glioblastoma (GBM; WHO grade IV) than to the prognosis of grade III tumors, in the WHO 2007 classification, GBM-O was introduced as a more formally accepted diagnosis. Subsequently, this change by the WHO put the controversial topic of GBM-O in the spotlight and multiple clinico-pathological studies were performed investigating the value of GBM-O as a separate group. These studies provided contradictory results and made it clear that histopathological distinction of GBM-O from other high-grade gliomas can be challenging because easily reproducible criteria are lacking. In an era that tumor classification is increasingly based on a combination of morphological and molecular information, it may well be that the diagnosis GBM-O is soon going to be replaced by a diagnosis of GBM, supplemented with information on the presence/absence of especially complete 1p/19q co-deletion and IDH1/IDH2 mutation.

Diagnosing gliomas by 450K methylation chip analysis
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Tumor classification in neuropathology currently is based on histology, immunohistochemistry and a selection of molecular markers. The observation that medulloblastomas can be divided into four meaningful biological subgroups by DNA methylation profiling prompted the speculation that other brain tumors may also be classified by their CpG methylation profile. To this end, we have created a methylation profile reference bank of nearly 2000 nervous system tumors and developed algorithms to rapidly compare new samples with this reference set. Our approach, dubbed “Methylation-Analysis,” is based on the analysis of approximately 200 ng of DNA derived from FFPE tissue. Our Methylation-Analysis-pipeline provides several highly valuable sets of information in a timely manner: – A classifier matches the respective sample with data from the cases in the reference set thus providing a best fit. This in many cases indicates a specific diagnosis and also allows further separation of established WHO entities into subsets of clinical relevance. – A copy number plot calculated from the data array provides valuable information such as 1p/19q losses, EGFR amplification and other chromosomal alterations. – The methylation status of genes such as MGMT is given. In conclusion, Methylation-Analysis represents a comprehensive tool for the validation of tumor diagnoses that provides information highly relevant for daily diagnostic work.

Histopathological classification of gliomas: about babies and bathwater
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Despite many decades of carefully implemented histopathologic studies, the current World Health Organization (WHO) scheme still utilizes subjective criteria for classifying and grading diffuse gliomas, which are inconsistently applied mainly on individual philosophies for distinguishing astrocytic and oligodendroglial lineages. As such, interobserver concordance rates are suboptimal, with diagnostic changes being common during outside pathology reviews for patient care and central reviews for clinical trials. Lastly, it has become progressively apparent that despite histologic similarities, pediatric gliomas differ biologically from their adult counterparts. This has led to huge frustrations among neuro-oncologists, with sometimes impassioned calls to completely abandon traditional light microscopy in favor of “objective” genetic analyses. Indeed, the last decade has brought remarkable advances, now allowing for incorporation of molecular data into glioma diagnosis. Nonetheless, we must avoid the temptation to leap too quickly into purely biomarker driven schemes that rely on insufficiently proven assumptions, ignore powerful and reproducible histopathologic parameters, or inappropriately combine newly fashioned molecular definitions of lineage with previously established morphology based grading criteria. Additionally, we should not forsake robust clinico-pathologic variables and cost-efficient diagnostic techniques in our haste to achieve greater interobserver reproducibility. In other words, as published in the 1512 “Appeal to Fools” by Thomas Munner, we must not “throw the baby out with the bathwater.” This lecture will highlight a recent international attempt to strike the appropriate balance, leading to published ISN-Haarlem consensus guidelines. Examples utilizing these guidelines in the diagnosis of adult gliomas will be presented.

S10: Updates in neurogenetics
Aiming for clinical sentinel signs in the search of genomic microaberrations
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In Medical Genetics, the presence of clinical sentinel signs has been a major semiology approach to guide for clinical, cytogentic, biochemical or molecular diagnosis. With the increasing emerging information from new laboratory genetics technology applied for clinical diagnosis, genomic microaberrations has been widely identified revealing new genetic disorders related to new clinical symptoms and/or signs. From birth defects to complex multifactorial diseases, a series of new related genes is now been unravel that reinforces the contiguous gene syndrome concept. Furthermore, the discovery of new genomic sites related to such genetic disorders identifies genes with uncertain clinical correlation to humans that should be addressed and translated in clinical grounds for patient management. Examples with therapeutic drug interventions (use of rhGH treatment), involvement of proto-oncogenes sites (screening for cancer), unknown methylated sites (growth control), as others, will be highlighted in this short presentation.

Pathologic autophagic processes integrating neurogenetic degenerative disorders
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Autophagy is an evolutionarily conserved and strictly regulated lysosomal pathway that degrades cytoplasmic material and organelles.
Three different autophagic routes are known: macroautophagy, microautophagy and chaperone-mediated autophagy. Since the discovery of autophagy-related genes in the 90s, many studies on the physiological and pathological roles of autophagy in a variety of autophagy knockout models have been undertaken. Only recently, though, direct evidence of the connections between autophagy genes dysfunction and human diseases has been shown. Many reports showing that mutations in the autophagy genes emerged in various human diseases such as neurodegenerative diseases, infectious diseases, and cancer.

It is not a surprise that mutations in genes regulating autophagy can cause neurodegenerative diseases. This is seen in relatively common disorders such as late-onset degenerative diseases (Alzheimer’s disease, amyotrophic lateral sclerosis and Parkinson's disease). The new aspects are that monogenic neurodegenerative disorders with early onset have been identified as genetic disorders of autophagy dysfunction; in such diseases, deficits occur at different stages of the autophagy pathway and have different implications for pathogenesis and therapy. Regarding therapy, pharmacological approaches to activate or inhibit autophagy are also important since autophagy can play either a protective or destructive role in many diseases, even in different stages of the same diseases.

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Spino cerebellar ataxias: polyglutamine diseases and beyond
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Spino cerebellar ataxias (SCAs) are a clinically homogeneous and genetically heterogeneous group of at least 37 autosomal dominant, late-onset disorders. Seven are caused by coding polyglutamines (polyQ) due to expansions of CAG repeats: SCA1, SCA2, SCA3/Machado Joseph disease (SCA3/MJD), SCA6, SCA7, SCA17 and dentato-rubro-pallido-luysian atrophy (DRPLA). Minimal prevalence of SCAs is 1.6 to 5.5:100,000. The relative frequency of each specific SCA varies among populations. For some reason, the polyQ SCAs are the most common, accounting for 50–90% of all families worldwide, and for 80% in Brazil. There is no causal therapy to prevent, cure, delay or slow down symptoms. Studies on pathogenesis and neuropathology are needed for developing effective treatments. The expanded CAG-repeats (CAGexp) are inversely related to disease severity, and the encoded protein with the polyQ tract seems to be the main reason for disease. PolyQ tracts mostly affect ubiquitous expressed proteins; however, their toxicity is limited to some neurons. Removing this toxic effect by RNA silencing might be a rationale treatment in future, but other treatments may play important roles. The CAGexp explains 30–70% of variation in age at onset; modifier factors related to the other 30–50% variation could be targeted. Drugs such as lithium, a pharmacological agent related to increased autophagy, increased chaperones, and HDAC inhibition, have been tested in SCA1, SCA2 and SCA3/MJD; other compounds and study designs are envisioned.

The overlap in disease phenotypes makes diagnosis dependent on molecular panels covering the majority of SCA loci, and reference centers prepared to receive samples from distant places were developed in Brazil and other countries. Finally, the lack of cure and the 50% risk to progeny raise the fundamental role of genetic counseling, including pre-symptomatic testing, in improving quality of life of SCA families.

The Schwann cell and the inherited neuropathies
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Myelinating Schwann cells (SC) are very important for the structure and function of the peripheral nervous system. During development successive SC enwraps the axon generating the myelin sheet. The space between two SC is the node of Ranvier a complex structure that concentrate the Na, channels that are essential for generating the action potentials, while the region between two successive nodes of Ranvier is the internodal region that has important function in the action potential propagation. This complex structure allows for the saltatory conduction that is essential for a fast transmission of impulses. Additionally, SC determine several characteristics of the axons, including axonal diameter, neurofilament spacing, neurofilament fotorilation, the position of Na, channels, and axonal regeneration after an injury.

Mutations in the genes coding for proteins of the SC and their prolongations or in proteins that are essential for myelin functions result in demyelinating inherited neuropathies. At least 16 different genes are now known to cause these neuropathies, including genes related to myelin assembly (PMP22, MPZ, PRX), membrane trafficking (MTMR2, MTMR13, MTMR5, NDRG1), transcription regulation (EGR2), mitochondrial dynamics (GDAPI), cytoskeletal remodeling (FGD4) and endocytic recycling (F4G, SH3T2). Interestingly, the severity of these neuropathies is dependent of the degree of the associated secondary axonal degeneration that is triggered by the myelin dysfunction.

The knowledge accumulated in the last decades made important contribution not only to the molecular diagnosis of the inherited motor and sensory neuropathies (CMT disease) but also to the understanding of the peripheral nerve biology, opening strategies for specific gene therapy for these patients.

Next-generation sequencing: the impact of new technology in research and diagnostics of neurological disorders
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After 2005, with the establishment and dissemination of next generation sequencing (NGS) methods, DNA sequencing costs start to drop quickly leading to a real revolution in the way we apply these methods to research and ultimately to genomic medicine. Currently, whole exome and whole genome sequencing have been used as a diagnostic test for different clinical conditions in different medical areas, including intellectual disability and other neurologic diseases with presumed genetic etiology. Although, the advantages of this new technology has been well appreciated, there are still a number of unresolved issues regarding its application in the clinical setting, among them the management of incidental findings and confidentially of genomic information, are leading causes of concern within the medical and scientific community. In addition, there are questions regarding the incorporation of these technologies in the public health sector, particularly in countries whose healthcare system is strongly supported by public investment, such as Brazil, United Kingdom, Canada, Australia, among others. All of these issues will be discussed in this presentation, together with the most recent applications of NGS technology in biomedical research.
S11: Autopsy contribution of neuropathology nowadays

Brain bank at University of São Paulo: a platform for histopathological correlation with magnetic resonance images using computerized methods to assess point-to-point correlation

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No biomarker is currently approved for clinical use to diagnose any of the common neurodegenerative diseases before symptom onset, by when a large percentage of brain tissue has already been irreversibly damaged. Magnetic resonance imaging (MRI) is the best tool available to study the human brain in vivo. Despite improvements in the past years, the maximum resolution of clinical MRI is still rough compared to histological examination. Since stronger magnetic fields are unlikely to be tolerated by humans, alternative methods are needed to close the gap between histology (post-mortem) and MRI examination. Since 2005, a multidisciplinary team has been using the Brain Bank of the Brazilian Aging Brain Study Group to develop a platform to correlate MRI and histopathological examination, point-to-point, aiming to (1) Brazilian Aging Brain Study Group to develop a platform to correlate MRI and histopathological analysis, point-to-point, aiming to (1) improve MRI sensitivity and specificity to smaller neuropathological changes, (2) expand the clinical use of MRI to monitor brain aging, diagnose neurodegenerative diseases, track disease progression and therapeutic efficacy, and (3) validate MRI signal to existing and novel modalities. This platform combines a highly innovative set of histological methods with advanced graphic computers to generate a database of perfectly aligned multimodal and multilevel brain maps derived from MRI and histology data. Currently, one case per week is being processed. The benefits of this approach goes beyond age-related problems. MRI use may be galvanized for other fields that are affected by MRI’s lack of resolution and validation, such as functional neurosurgery, epilepsy treatment, neuro-oncology, and even to other organs.

The influence of age and sex in the absolute cell composition of the human brain

Lent R

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Cellularity in the human brain can be studied with a novel technique, the isotopic fractionator that transforms its anisotropic structure into a homogeneous suspension of cell nuclei, which can then be precisely quantified at the microscope. We first studied brains of normal middle-aged human males, showing that they do not hold one-hundred billion neurons and 10-fold glial cells as usually believed, but 15% less, plus an equal amount of non-neuronal cells. We then investigated whether normal aging would affect cell numbers, focusing on the cerebellum and hippocampal formation of females 25–92 years old. While no change was found in the cerebellum, a slight but significant drop of neuronal numbers occurred in the hippocampus, plus an increase in glial numbers. We next questioned whether Alzheimer’s disease (AD) imposes changes on brain cellularity. Three groups were studied: subjects cognitively and histopathologically normal; patients with severe dementia and postmortem diagnosis of AD; and a group with asymptomatic AD. We found a great reduction of neuronal numbers in the hippocampus and cerebral cortex of demented patients with AD, but not of asymptomatic AD subjects, therefore concluding that neuronal loss is associated with dementia, not with plaques and tangles. Finally, we studied the influence of sex on cellularity of brain regions. Results showed a significant sexual dimorphism in the olfactory bulb, favoring women with over 40% more neurons and glial cells than men; and in the hippocampal formation, favoring men with over 30% more neurons and glial cells. No sex-difference was observed in the cerebellum.

The experience of Virginia University

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Autopsy rates have been steadily decreasing worldwide in the last decades. In the USA, autopsies account for only 5–8% of hospital deaths. Nevertheless, the valuable role of autopsies for quality assurance in medical practice and epidemiologic and population studies are unquestionable. Moreover, autopsy pathology plays a significant role in pathology training for medical students and most significant pathology residents and fellows. Although autopsy pathology is an essential part of the Residency Training Programs in the USA, due to the significant decrease of hospital-based autopsies, the American Board of Pathology has over the years decreased the minimal number of autopsies performed by a training prior his/her graduation, with a minimum of 50 cases during the 4-year program. Similarly, the minimal number of brain autopsies analyzed by a fellow in an accredited Neuropathology Fellowship Program in the USA has decreased to a minimum of 150 cases during the 2-year training program. The difficulties to achieve the minimum number of brain autopsies for a Neuropathology Training Program in a relative small university hospital as the University of Virginia, led us to invest in a consultation based network of brain sources including the Virginia State Medical Examiner system, community hospitals, and the National Prion Surveillance Center.

Contribution of a small brain bank to the knowledge of CNS diseases

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Brain banking is a necessary activity nowadays in the field of neuropathology-neuroscience, and is usually devoted to collect brains either for certain diseases, as a part of longitudinal studies, or as a part of a diagnostic service in order to provide nervous tissue to researchers. The “income” of a brain bank depends on the number of autopsies entering into the service or the study, and that number usually depends on many factors such as social-cultural, how rare the disease is, or the existence of a previously established longitudinal study. In the case of our brain bank in Argentina, even though still small, we’ve been collecting brains and providing tissue to researchers since the beginning, and probably, the biggest contribution so far has been the detection and description of previously unrecognized diseases among our population.

Brain banking of the 21st century – creative solutions and ongoing challenges

Ravid R

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Brain banking organizations who systematically collect and supply clinically and neuropathologically well-documented specimens are essential for high-quality research into neurological and psychiatric disorders. The growing number of sophisticated neurobiological techniques which can be applied to human specimens obtained from donors at autopsy has increased the pressure on neuropathologists and brain banks (BBs) to provide tissue for research conducted within the...
scientific community and pharmaceutical companies. Active BBs form an important link between tissue donors, their relatives, clinicians, neuropathologists, and scientists. The most successful model proves to be a combination of a local donor system and informed consent, combined with rapid autopsy and fresh dissection protocols for each disease, based on the specific pathological hallmarks. BBs are seeking to reach a consensus on the clinical and neuropathological diagnostic criteria, which will make their specimens suitable for high-quality research. The benefits and spin off will be considerable, as improved understanding of neurological and psychiatric disorders will contribute significantly to the elucidation of their underlying disease mechanisms, and this has the potential to identify rational therapeutic targets.

S12: Stem cells in neurosciences: iPS cell and neuropathology
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This conference will focus on insights gained through the use of human pluripotent stem cell preparations regarding modeling mental disorders in vitro, including the roles of oxidative stress and atomic element imbalance in schizophrenia. Translational efforts in academic and industry to use stem cell microsystems and mini brains as approaches for drug screening, personalized and regenerative medicine will be highlighted.

Clinical trials of stem cells for ischemic stroke
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Stroke is the second leading cause of death and the third leading cause of disability worldwide. Approximately 16 million first-ever strokes occur each year, leading to nearly 6 million deaths. Nevertheless, currently, very few therapeutic options are available. Cell therapies have been applied successfully in different hematological diseases, and are currently being investigated for treating ischemic heart disease, with promising results. Recent preclinical studies have indicated that cell therapies may provide structural and functional benefits after stroke. However, the effects of these treatments are not yet fully understood and are the subject of continuing investigation. Meanwhile, different clinical trials for stroke, the majority of them small, nonrandomized, and uncontrolled, have been reported, and their results indicate that cell therapy seems safe and feasible in these conditions. In the last 2 years, the number of published and registered trials has dramatically increased. We will review the main findings available in the field, with emphasis on the clinical results. Moreover, we address some of the questions that have been raised to date, to improve future studies.

IPS cells for neurological diseases
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Parkinson’s disease (PD) is a neurodegenerative disorder mainly characterized by the degeneration of dopaminergic neurons of the substantia nigra pars compacta (SN), leading to a dysfunctional nigrostriatal pathway. Transplantation of dopamine-producing cells into the striatum has already shown its effectiveness in animal models and clinical trials, although undesired complications in a number of cases and the lack of a homogenous and GMP-grade cell preparation precludes its wide application in the clinic. Another major caveat of this approach is the low level of cell survival after transplantation. We sought to test the effects of the striatal transplantation of human, GMP-grade dopaminergic neurons derived from embryonic stem cells into rat models of Parkinson’s disease. Adult rats were inoculated with 6-OHDA, tested for motor asymmetry and transplanted with $8 \times 10^4$ dopaminergic neurons (DA) or control cells into the striatum. Control cells were human pluripotent stem cells subjected to a similar differentiation protocol as the DA cells, but harvested 4 days before they start producing dopamine. Animals were immunosuppressed with cyclosporine A 1 day before and throughout the experiment. Pharmacological and non-pharmacological tests showed a clear improvement in motor behavior of animals transplanted with DA cells but not the control cells 12–13 weeks after transplantation. Persistent microglial activation at the site of transplantation was observed by MHC-II, ED-1 and GSA staining 90 days post-transplantation in DA 14 and control cells-treated animals. Similar experiments are being conducted in an inflammation-based animal model of PD.

We conclude that the transplant of this GMP-grade preparation of human DA neurons provides a robust motor recovery in the animals tested. In addition, the chronic inflammation observed at the site of transplantation opens up the possibility of increasing neuronal survival and differentiation by immunomodulation.

Cell therapy in neurological diseases
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Neurological diseases have a high prevalence, mortality and social cost both in developed and developing countries. In Brazil, cerebrovascular diseases are the leading cause of death with approximately 90,000 deaths/year and effective pharmacological therapies are unavailable. In this context, stem cells have been considered as potential therapies but many questions must be answered before entering clinical practice. In this presentation, we will show the results of our studies in which we have used animal models of neurological diseases in order to investigate the therapeutic role of stem cells. In these studies, we were able to show that cell therapy with bone marrow derived cells decreases brain injury and increases functional recovery after focal ischemia. The mechanisms of action involved in the beneficial effect of bone marrow derived cells seem to be related to paracrine release of trophic and/or neuroprotective factors, which decrease neuronal death and/or modulate microglia reactivity. We have also investigated the biodistribution and therapeutic effects of the cells delivered using different markers. The observations from the preclinical studies were translated to a few clinical studies to assess the safety and feasibility of transplantation of autologous bone marrow derived cells in patients with middle cerebral artery ischemic stroke. We will discuss future possible clinical studies to assess the efficacy of this treatment approach.

Patient-derived IPS cells in the study of neurological diseases
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In this presentation, we will review the use of cellular reprogramming and patient-derived cell lines to study disease mechanisms in neurological disorders. The focus of this discussion will be neuromuscular disorders associated with dysfunction of the spinal motor neuron (motor neuronopathies) and itsaxon (axonopathies), which are severe conditions with no effective treatment available. Findings from animal models have so far translated poorly in clinical trials, underscoring the need for innovative methods to investigate the pathophysiology of these human disorders. Induced pluripotent stem cells (iPSC) offer an unlimited source of patient specific, disease-relevant cell lines that can be used as a platform for identification of disease mechanisms, discovery of molecular targets and development of phenotypic screens for drug discovery. iPSC-based models of neuromuscular disorders, including amyotrophic lateral sclerosis (ALS), spinal muscular atrophy (SMA) and axonal Charcot-Marie-Tooth disease (CMT), have success-
fully reproduced pathophysiological findings from animal and other cellular models and have also identified new disease mechanisms with potential therapeutic implications. This model is amenable to interrogation by multiple techniques, including gene expression profiling, time-lapse microscopy, single-cell and multi-array electrophysiology and electron microscopy. The ability of this approach to detect treatment effects from known therapeutic compounds has also been demonstrated, providing proof of principle for the use of iPSC-derived cells in drug discovery. In summary, cellular reprogramming offers a new approach for the study of neurological disorders by making it possible to study human cell types directly related to their pathophysiology (neurons and glia) and to reproduce neuronal development in vitro.

LS5: Nonspecific tauopathies

Why is it relevant to distinguish astroglial pathologies in tauopathies?

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Intracytoplasmic glial deposits of hyper-phosphorylated tau are common, but the majority of, if not all, sporadic and familial tauopathies. In contrast to oligodendrogial inclusions, which are most commonly manifested as coiled and globular bodies, astroglial deposits were first classified according to their morphology sometimes characteristic, although not specific, of a particular disease as tufted astrocytes in progressive supranuclear palsy, astrocytic plaques in corticobasal degeneration and diffuse granular hyper-phosphorylated tau immunoreactivity of astrocytic processes in the elderly. Later on, it was suggested that astroglial phenotypes depended on (i) the primary amino acid sequence of tau (mutated tau, 3Rtau and 4Rtau); (ii) phospho-specific sites of tau phosphorylation, tau conformation followed by tau truncation and ubiquitination, which define pre-tangle and tangle stages, respectively; and (iii) modifications of astroglial cytoskeleton. Molecular characteristics of astroglial deposits resemble but are not identical to those of pre-tangle and neurofibrillary tangle neurons in the same tauopathy. Identification of astroglial subtypes in different brain regions in normal brains is probably necessary to understand their feasible particular responses in different tauopathies. Hyper-phosphorylated tau inclusions may have functional implications as reduced expression of glial glutamate transporter thus hampering glutamate transport. Finally, unique astroglial pathology can reveal new familial tauopathies not linked to mutations in the tau gene.

A novel motor neuron disease and frontotemporal lobar degeneration with globular astrocyte-predominant tau and TDP-43 inclusions

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Amyotrophic lateral sclerosis, which is a major TDP-43 proteinopathy, is sometimes accompanied by dementia (ALS-D). We report an unusual tauopathy in a Japanese patient who was diagnosed clinically as having ALS-D. Autopsy examination revealed frontotemporal lobar degeneration (FTLD) with the most severe neuronal loss and gliosis in the precentral cortex. Although less severe, such changes were also observed in the other brain regions, including the basal ganglia and substantia nigra. The subcortical white matter of the motor area was severely rarefied with myelin pallor. In the spinal cord, loss of anterior horn cells and degeneration of the corticospinal tracts were evident. Immunostaining using an antibody against hyperphosphorylated tau revealed widespread occurrence of neuronal and glial cytoplasmic inclusions in the central nervous system. The astrocytic tau lesions were those of globular astrocytic inclusions (GAls), being different in morphology from astrocytic plaques in corticobasal degeneration (CBD) or tufted astrocytes in progressive supranuclear palsy. A few globular oligodendrocytic inclusions were also observed in each affected region. Of great interest was that although partially, the astrocytic lesions were also immunoreactive with an antibody against phosphorylated TDP-43. Immunoblotting of frozen brain samples revealed predominantly 4-repeat (4R) tau, with the approximately 37-kDa low-molecular mass tau fragments characteristic of CBD. No mutations were found in the MAPT and TDP-43 genes. These findings suggest that this patient forms a distinct globular glial 4R tau and TDP-43 proteinopathy in association with MND and FTLD.

The spectrum of tau-astrogliopathies in the elderly: an attempt for classification

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In the ageing brain, the most frequent alteration is the neurofibrillary degeneration with or without senile plaques, and argyrophil granules. Widespread application of phospho-tau immunohistochemistry revealed further tau pathologies and constellations of these. Unfortunately, there is a lack of consensus as to how these can be grouped or even what is the exact clinical relevance of these alterations. However, as a common feature tau astrogliopathy can be mentioned, which shows different morphologies and anatomical distribution. These tau pathologies may have variable influence on clinical presentations, depending on the presence of concomitant pathology and the individual's threshold for the development of clinical symptoms. Different descriptions most likely represent a spectrum or stages of similar conditions coined with different terms. From a practical aspect, based on our and others experience, the following patterns can be recognized: (1) Gallyas negative astrocytes with granular tau immunoreactivity in the processes in the medial temporal lobe grey matter associated with a few, mostly argyrophilic thorny astrocytes in the white matter and more in the subpial and subependymal region without overt symptoms of dementia; (2) rarely, prominent tau-astroglialopathy (either granular in processes, thorny and tufted-like) is restricted to the amygdala; (3) in some cases, the tau-astroglialopathy expands beyond the prominently affected medial temporal lobe and is associated with neuronal tau pathology, including pretangles in limbic cortices, basal ganglia, and substantia nigra.
of neurons. Finally, intracerebroventricular injection of A25T fibrils activated microglia causes synapse loss culminating in apoptosis of microglia. Exposure of neuronal cultures to conditioned media of A25T amyloid fibrils induce NF-κB and cause microgliosis and cognitive deficits in mice, which could be caused by fibrillation of A25T-TTR. We have showed that fibrils of A25T activate microglia leading to secretion of TNF-α, IL-6 and nitric oxide. A25T amyloid fibrils induce NFXβ translocation to the nucleus of microglia. Exposure of neuronal cultures to conditioned media of fibril-activated microglia causes synapse loss culminating in apoptosis of neurons. Finally, intracerebroventricular injection of A25T fibrils caused microgliosis and cognitive deficits in mice, which could be prevented by minocycline. These results indicate that A25T fibrils act as inflammatory agents, activating microglia and neuronal death.

Neuropathology of TTR familial amyloid neuropathy
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Familial amyloid neuropathy (FAP) is a rare autosomal dominant disease first described in Portugal by Andrade in 1952. It is a progressive fatal disease with onset between the age of 20–30 years resulting from extracellular deposition of β-sheeted amyloid fibrils mainly in the peripheral nerves, causing a severe sensory, motor and autonomic neuropathy, initially affecting unmyelinated and small diameter myelinated fibers. The most common mutation is where methionine replaces valine in position 30 (Val30Met). There are 664 families with 2657 patients mainly in Northern Portugal, 51% of the cases were diagnosed in the last two decades. The prevalence in Portugal is 1/1000 being an endemic disease.

The pathology consists on the deposition of amyloid in the connective tissue mainly in the peripheral nerves, dorsal root ganglia and the autonomic nervous system. CNS is also affected with leptomeningeal and choroid plexus. Over the last 40 years, around 1000 nerve, skin and salivary glands biopsies were studied and 30 autopsies were also performed in our department. Before molecular diagnosis of FAP was available, demonstration of amyloid deposition was required for diagnosis. Skin biopsies are almost as reliable as nerve but now salivary gland biopsies, a minimally invasive procedure, is the method of choice in our center to demonstrate and identify the type of amyloid, necessary to start treatment with the new amyloid stabilizer drugs or to be submitted to a liver transplant.

Current insights into quantifying transthyretin aggregation-associated pathology and its amelioration with tafamidis
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Recently, we showed that tafamidis, a small molecule kinetic stabilizer of transthyretin, dramatically slows the progression of familial amyloid polyneuropathy in a placebo controlled clinical trial of 18-month duration (1), and in a 12-month follow-on open label study (2). This

Recent advances in clinical and therapeutic approaches to FAP
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Familial amyloid polyneuropathy (FAP) is a dominant inherited amyloidosis caused by the deposit of one of more than 100 already described variants of TTR. TTR is a homo tetrameric, plasmatemic protein mainly produced in the liver (98%). In Brazil, the most common mutation found is TTRV30M and the disease presents mostly as an early onset peripheral neuropathy affecting small nerve fibers progressing to a complete autonomic, sensory and motor neuropathy. FAP is also a systemic disease affecting cardiac and renal function. Liver transplantation has been the current standard of care for TTR FAP patients since it ceases the production of the mutant TTR by the liver which reduces disease progression. However, there are still concerns about mortality and morbidity related to the procedure itself and the immunosuppression that follows and the availability of the organ.

More recently, the development of drugs that stabilize the TTR tetrameric structure through the binding into the thyroxine channels, such as tafamidis and diflunisal, has been shown to be effective to slow disease progression. In a recently concluded controlled trial, patients treated with tafamidis had numerically smaller increases in Neuropathy Impairment Score-Lower Limb (NIS-LL) and Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN) scores at 66 months. Tafamidis was generally well-tolerated. In another well-controlled finished trial diffusion also proved to be effective in slowing disease progression but there is concern about long term safety use of this drug.

Other promising strategies are under study such as patisiran which comprises a small interfering ribonucleic acid (siRNA), which is specific for TTR, and is formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration. This TTR siRNA has a target region within the 3’UTR region of TTR gene to ensure and confirm homology with WT TTR as well as all reported TTR mutations. Following LNP-mediated delivery to the liver, patisiran targets TTR mRNA for degradation, resulting in the potent and sustained reduction of mutant and WT TTR protein via the RNAi mechanism. Also, ISIS-TTRRx, is an antisense oligonucleotide that targets specific sites at RNA inhibiting RNA function leading to no translation of disease causing protein TTR.ISIS-TTR Rx is delivered SC. Strategy of both Patisiran and ISIS-TTRRx is to decrease the amount of wild-type and mutant TTR circulating in the plasma. It is predicted that those drugs would reduce further fibril formation and slow or halt disease progression. Both drugs are potentially more robust strategy compared to liver transplant as liver transplant removes mutant TTR, but wild type TTR continues to deposit most likely limiting therapeutic response.

LS6: TTR familial amyloidotic neuropathy: from pathology to treatment
The role of microglia and neutrophils in transthyretin (TTR)-related amyloidoses
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Neutrophil extracellular traps (NETs) are key players in a death mechanism in which neutrophils release DNA traps decorated with elastase and histones to entangle pathogens. We showed that amyloid fibrils from three different sources (st-synuclein, Sup33 and transthyretin) induced NADPH oxidase-dependent NETs in vitro from human neutrophils. Surprisingly, NET-associate elastase digested amyloid fibrils into short species that were cytotoxic for BHK-21 and HepG2 cells. We observed in situ, NETs in amyloidotic deposits from amyloidotic patients which co-localized with amyloid deposits. These data reveal that NETs can be triggered by amyloid fibrils. Moreover, the involvement of NETs in amyloidoses might be crucial for the production of toxic species. In a second approach, we have investigated the pathogenic mechanism behind oculoleptomeningeal amyloidosis (OA) caused by fibrillation of A25T-TTR. We have showed that fibrils of A25T activate microglia leading to secretion of TNF-α, IL-6 and nitric oxide. A25T amyloid fibrils induce NFXβ translocation to the nucleus of microglia. Exposure of neuronal cultures to conditioned media of fibril-activated microglia causes synapse loss culminating in apoptosis of neurons. Finally, intracerebroventricular injection of A25T fibrils caused microgliosis and cognitive deficits in mice, which could be prevented by minocycline. These results indicate that A25T fibrils act as inflammatory agents, activating microglia and neuronal death.
study presents the first pharmacologic evidence supporting the hypothesis that the process of active transthyretin aggregation causes the degeneration of post-mitotic tissue (i.e. the peripheral nervous system, the autonomous nervous system, and the heart) in the human amyloidoses. To further test the hypothesis that active TTR aggregation causes post-mitotic tissue degeneration, we developed five new clinical assays to measure tafamidis plasma concentration, to quantify tafamidis-associated kinetic stabilization of tetrameric transthyretin, to determine the concentration of soluble misfolded TTR levels, to quantify natively folded TTR tetramer levels in plasma, and to quantify the pathological gene expression profile in peripheral plasma cells of familial amyloid polyneuropathy patients. The results of these assays before and after dosing Portuguese and Japanese patients with tafamidis post-regulatory agency approval will be presented.

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FAP in Latin America and Brazil: an overview
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Introduction: The natural course of transthyretin-related amyloidosis (ATTR) is poorly defined due to the extreme heterogeneity in genotype and phenotype as well as the relatively low disease prevalence. The global, non-interventional THAOS patient registry was established in 2007 to collect and analyze data on symptom occurrence and progression and on the effects of disease modifying treatments in a large, diverse patient population. This current analysis provides a Latin-American perspective on the THAOS dataset.

Methods: THAOS is an ongoing, longitudinal, observational registry open to all symptomatic individuals with confirmed hereditary or acquired ATTR, as well as to asymptomatic carriers of known pathogenic TTR mutations. Patient data from a range of standard assessments are obtained during clinical evaluations and recorded using an interactive Web-based system.

Results: As of June 2013, a total of 1744 subjects from 17 different countries were enrolled in THAOS, with 64 different TTR genotypes. Most patients from Brazil (94/105) and Argentina (38/40) carried the Val30Met mutation, whereas the Ser50Arg mutation (27/34) was predominant in Mexico. Certain genotypes were generally associated with predominantly neuropathic (e.g. Val30Met) or predominantly cardiac (e.g. Val122Ileor wild-type) symptoms. Analysis of the prevalence of ATTR symptoms in patients from major genotype groups categorized by the duration of their symptoms provides insights into the natural course of ATTR.

Conclusions: The large dataset from the THAOS registry presents a unique opportunity to improve our understanding of the diverse presentations and the natural disease progression of ATTR.

Disclosures: The THAOS registry is sponsored by Pfizer Inc.

W9: Novel concepts of meningioma biology and translational neuropathology research

General pathology and new molecular biology features
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Accounting for up to 35% of all primary CNS neoplasms, meningioma is one of the most common diagnoses encountered in neurosurgical practice. It has long been appreciated that the two most important prognostic variables for meningioma patients are extent of surgical resection and histopathologic grade. Grading criteria have varied greatly over time, with the current 2007 WHO scheme largely derived from data reported in two large Mayo Clinic series published in the late 1990s. Since that time, the clinicopathologic associations have been independently validated, despite some concerns that the grade II category has become too commonplace. Of interest, a recent RTOG clinical trial revealed home pathology/central pathology concordance rates of 86.7%, 81.7%, and 94.0% for WHO grades I, II, and III, respectively, suggesting substantial agreement for overall grade and moderate to substantial agreement over individual grading criteria. The derivation of the current grading scheme and remaining controversies will be discussed, along with the remaining need to develop better diagnostic, prognostic, and predictive biomarkers for routine clinical practice. Two potentially sensitive and specific markers that were recently reported include somatostatin receptor 2A (SSTR2A) and STAT6 immunostains for diagnoses of meningioma and heman-giopericytoma/solitary fibrous tumor respectively. Additionally, genetic advances beyond the role of the NF2 gene will be highlighted briefly. These include the recently identified driver mutations of TRAF7, KLF4, AKT, and SMO genes, along with studies of progression associated alterations, such as CDKN2A gene deletions on chromosome 9.

Update and future directions on meningioma mouse models
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We have showed that
(1) NF2 gene inactivation in arachnoidal cells is rate-limiting for meningioma development in the mouse using arachnoidal cell adenovirus Cre-mediated inactivation of NF2.
(2) Additional hemizygosity for p53 does not modify meningioma frequency or progression suggesting that NF2 and p53 mutations do not synergize in meningioma tumorigenesis.
(3) In addition to bi-allelic NF2 inactivation, homozygous Cdkn2ab deletion leads to increased meningioma frequency with a shortened latency and induces grade II/III meningioma development suggesting that NF2 and Cdkn2ab cooperate to promote meningioma progression in mice.
(4) Prostaglandin D synthase-positive meningeal precursor is the cell of origin capable of generating diverse meningioma histological subtypes. Using a unique PGDSCre strain, we defined a critical embryonic and early postnatal developmental window in which biallelic NF2 inactivation in PGDS (+) progenitor cells results in meningioma formation.

Using somatic cell type-specific gene transfer, we investigate the role of PDGF signaling in meningiogenesis by transferring the overexpressed PDGF-B into PGDS+ meningeal cells. We have observed that overexpression of PDGF-B induces meningioma development in mice and that additional NF2 and Cdkn2ab inactivation enhance malignancy of meningiomas. We are currently analyzing the role of AKT1(E17K) and SMO (Hedgehog signaling) mutations in meningioma development to understand
Uncovering mitogenic signaling in meningiomas as a basis for future drug trials
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Meningiomas are the most frequent intracranial tumors, and current treatment options include neurosurgery and radiation therapy. A significant fraction of meningiomas, however, will recur or may need additional treatment options. The use of conventional chemotherapy, for instance hydroxyurea, has been of limited success. Therefore, modern targeted therapies might open a reasonable strategy to treat meningiomas. Detailed in vitro studies using meningioma cell culture systems, as well as investigations using human meningioma tumor tissue have demonstrated activation and functional relevance of a broad range of signaling pathways. Oncogenic signaling is activated mainly by receptor tyrosine kinases (RTK) such as PDGF-R, IGF-R, and EGF-R. Receptor activation drives growth-promoting intracellular signaling cascades, mainly the PI3-kinase and MAPK pathways. This results in cell cycle deregulation, increased protein biosynthesis (mTOR), generation of lipids for tumor cell membranes (fatty acid synthase), and increased glycolysis turnover (G6P). All components of these signaling pathways are reasonable targets for the treatment of unresectable and/or recurrent meningiomas.

W10: Updates on demyelinating diseases
MicroRNA changes in multiple sclerosis
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Micro RNAs are short non-coding RNAs, which play a major role in normal development, health and disease, by regulating gene function. Multiple pathological processes play a role in the pathogenesis of the lesions found in multiple sclerosis, some of which are generic and others of which may be more specific to the disease. These processes include dysregulation of the immune system, axonal and myelin injury, blood-brain barrier dysfunction, as well as repair. Identification of changes in the microRNA profiles may offer some clues as to potential functional gene and protein targets of these miRs, and thus will help in the identification of potential therapies directed toward these functions. Changes in microRNAs may be detected in tissue, blood and bone marrow and lymphoid derived lymphocytes and monocytes. microRNA profiles have been compiled, and these have shown differences between patients and controls, both in the lesions and the NAWM, as well as between different sub-sets of the disease. Many of these changes may also be documented in EAE (experimental allergic encephalomyelitis). We, and others, have used laser capture of specifically-identified cell types to identify the microRNA profiles in these cells found in both acute and chronic lesions. Differences have been detected in numerous microRNAs, of which miR-155 is among the most striking, especially noted with differential polarization in macrophage activation, associated with pro-inflammatory destruction and anti-inflammatory repair. CNS microglia and bone-marrow derived macrophages, identified by new differential antibodies also show differential changes in the disease. These miRNA changes are also noted to be altered in other cell types.

Demyelination after discovery of aquaporin 4 (multiple sclerosis, neuromyelitis optica)
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Since the first report of neuromyelitis optica (NMO) more than a century ago, the relation between NMO and multiple sclerosis (MS) has long been debated. The discovery of NMO-specific aquaporin-4 (AQP4)-IgG has accelerated clinical and experimental research of NMO and clarified the differences between NMO and MS. The clinical spectrum of NMO is now considered wider than severe optic neuritis in the original descriptions, and the International Panel on NMO Diagnosis is to publish the new diagnostic criteria of “NMO spectrum disorders (NMOSD),” which is the unifying term for this entity. Meanwhile, even now, in the majority of NMO-related papers and book chapters, the first sentence of introduction is “NMO is a severe inflammatory demyelinating disease of the central nervous system.” However, “demyelinating disease” should perhaps not encompass all diseases that cause myelin destruction but include only the ones in which damage of myelin is more severe than that of neuron. Accumulated pathological and experimental evidences strongly suggest that astrocytic damage is the primary pathology and is far more severe than damages of myelin and neuron in NMOSD. Hence, we proposed that NMOSD should be classified into a new entity of “autoimmune astrocytopathic disease.”

On another front, there exist patients with clinical features of NMOSD who are consistently AQP4-IgG-seronegative despite the use of the most sensitive assays, and among those with AQP4-IgG-seronegative NMOSD, some are seropositive for myelin oligodendrocyte glycoprotein (MOG)-IgG. Unlike AQP4-IgG-positive NMOSD, MOG-IgG-positive NMOSD is probably a demyelinating disease. Thus, NMOSD contains two distinct categories of diseases.

Where has all the white matter gone?
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Disorders in which cavitation or cystic degeneration of central white matter is exclusive or predominant are rare and of diverse etiologies. There are two pathogenetic types: cavitary and cavitating. The former is a static lesion, usually caused by a single necrotizing insult that overwhelms both axons and glia, such as perinatal ischemia. The latter is more dynamic and due to a multiphasic or chronic progressive degeneration of white matter that may primarily affect myelin, axons, glia, or blood vessels, such as leukodystrophies. In both types, supratentorial white matter is most severely affected, where severe axonal loss and an inadequate astrocytic response are necessary for cavitation to occur.

In spite of considerable axonal loss in most leukodystrophies, cavitation is typically seen only in infantile Alexander’s disease and vanishing white matter disease (VWM). In both, morphologically abnormal astrocytes are conspicuous, implying a defective astrocytic response. Functional studies confirm this. VWM, due to mutations in elf1 (eukaryotic initiation factor) 2B, is one of the most prevalent causes of cavitation. The most severe cavitation is usually seen in the infantile-onset patients, but exceptions occur. Longitudinal MRI studies have provided insights into this cavitation, but have not answered why a defective universal housekeeping gene should manifest exclusively as a white matter disease. Recent neuropathologic investigations reveal roles for (1) activation of the unfolded protein response in white matter glia, resulting in concomitant and contradictory expression of proliferative, prosurvival and proapoptotic downstream effectors; (2) abnormal maturation of white matter glia; and (3) possible interference in the maturation of oligodendroglial progenitors by high molecular weight hyaluronan.

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Azetidine-induced oligodendroglialopathy: relevance to multiple sclerosis
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The “cause” of MS is unknown. The non-protein imino acid Azetidine-2-carboxylic acid (Aze) is abundant in sugar beets (SB). After sugar extraction, SB byproducts are fed to livestock, particularly dairy cows. The history and spread of SB agriculture and entry of Aze into the food chain parallel MS history and global epidemiology. Rubenstein (2008) hypothesized that early life exposure to Aze results in substitution (misincorporation) for proline in human oligodendrocytes (OGC) and myelin proteins, (which are stable throughout life). Aze induces myelin protein misfolding in vitro.

To assess Aze effects on the CNS in vivo, pregnant females and newborn CD-1 mice pups were given Aze or saline po. Juvenile mice were given Aze ip or po (gavage) qd or saline. High-dose Aze induced systemic toxicity. High and moderate lower Aze doses induced dose-dependent OGC nuclear swelling throughout CNS white matter and OGC apoptosis (TUNEL/caspase-3 IHC). There was multifocal microglial activation but no detectable effects on other CNS cells or on myelin. OGC nuclear swelling and apoptosis (Prineas, 2012), and focal microglial activation are found in MS patient normal-appearing white matter (NAWM) and adjacent to active lesions. Thus, Aze-induced oligodendroglialopathy models OGC alterations and tissue responses found in MS patient NAWM. In humans, Aze misincorporation during CNS myelination might manifest later as enhanced vulnerability to OGC stress leading to the degeneration that underlies MS progression. Because of the historical and epidemiologic correlations of SB agriculture and MS, early life dietary Aze exposure may be a factor contributing to MS pathogenesis.

Meningeal inflammation and the molecular mechanisms of cortical pathology in multiple sclerosis
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The extensive pathology seen in the cortical grey matter is suggested to play a major role in the slow neurological deterioration during progressive MS. Although it has been suggested that inflammation plays a lesser part in the pathology of progressive MS, our data on over 480 brains in the MS Tissue Bank at Imperial shows a clear association between inflammatory activity and clinical progression. Our studies of a large cohort of cases reveals that the presence of inflammatory infiltrates in the leptomeninges, both diffuse and terephral lymphoid like structures, associates with increased demyelination, microglial activation, a gradient of neuronal loss and shorter disease duration. Analysis of meningeal gene expression of these cases reveals an increase in the expression of proinflammatory and cytotoxic cytokines (TNF, lymphotixin, interferon gamma) and chemokines involved in T and B cell recruitment (CXCL9, CXCL13). Analysis of gene expression in the underlying cortical GM shows that there is an imbalance in the TNF signalling pathways such that the TNF/TNFFR1 pathways via RIP1/RIP3 leading to necrotic cell death are increased in cases with high meningeal inflammation, whereas the TNF/TNFFR2 protective pathways via TRAF2/TAK1/NFkB are upregulated in cases with less meningeal inflammation and reduced GM pathology. These results suggest that changes in the balance of TNF signalling as a result of meningeal infiltration play a major role in cortical GM pathology in MS.

W11: Toxic neuropathology
Neurotoxic condition caused by aluminium adjuvants of vaccines: macrophagic myofasciitis
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Aluminum oxyhydroxide (alum), a nanocrystalline compound forming agglomerates, has been widely used as an adjuvant of vaccines since 1927. The mechanisms by which it stimulates immune responses remain poorly understood. Although generally well tolerated, alum may occasionally cause chronic health problems in presumably susceptible individuals. Some individuals may present with delayed onset of diffuse myalgia, chronic exhaustion and cognitive dysfunction, associated with long-term persistence (up to ears) of alum-loaded macrophages at site of i.m immunizations, forming the so-called macrophagic myofasciitis (MMF). Symptoms are consistent with the chronic fatigue/myalgic encephalitis (CFS/ME) syndrome, and were used as a paradigm of “autoimmune/inflammatory syndrome induced by adjuvants” (ASIA). Cognitive dysfunction is reminiscent of that described in workers exposed to inhaled Al particles. Individual susceptibility factors may influence both alum biopersistence and diffusion away from injection sites. Biopersistent particles, e.g. fluorescent alum-coated nanohybrids, injected into mouse muscle are captured by monocyte-lineage cells and then carried to distant organs, to draining lymph nodes, and then to blood, probably via the thoracic duct, with delayed and accumulative translocation to brain (microglial cells). Brain penetration occurs at extremely low level in normal conditions which is consistent with good tolerance to alum despite its high neurotoxic potential. However, systemic diffusion of particles considerably increase under the potentiating effect of MCP-1, the main monocyte chemoattractant factor, which production is subjected to important variations linked to age, genetic, and environmental factors. Selective MCP-1 increase is the sole circulating biomarker in MMF patients.

Toxic peripheral neuropathies
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Alcoholic polyneuropathy (PN) is characterized by subacute or chronic axonal degeneration and loss of myelinated and unmymelinated axons. Similar lesions are observed in the “tropical neuropathies.” Medication-induced PN have long been known for well-established drugs such as amiodarone and chloroquine. The lesions are similar: demyelination and presence on EM examination of numerous polymorphous inclusions assuming a lamellar or pseudomyelinic appearance in any cell cytoplasm. Such lesions are also found in muscle. This abnormal accumulation of lipid material takes a long time to disappear after the drug has been withdrawn.

The nerve lesions of other neurotoxic drugs (mostly chemotherapy-induced toxic PN (CIPN) are increasing; platinum compounds, vinca alkaloids, taxanes, bortezomb and thalidomide exhibit non-specific axonal involvement (length-dependent PN), which may be difficult to distinguish from lesions induced by the disease for which they have been prescribed. Cessation or diminution of the treatment usually stops progression of CIPN, except for platinum compounds: symptoms and signs may still progress or at least persist for several months. Several industrial chemicals can lead to PN: lead, arsenic, mercury, thallium. Also, many petroleum derivatives: solvents, gas, pesticides/herbicides and plastics may induce PN: acrylamide, organophosphates, trichlorehylene, DDt, tetrachlorehane, pentachlorophenol. They still may be seen in emerging countries where environmental pollution and occupational exposure are poorly controlled. For many of them, pathological lesions have not been described; nevertheless, it
is well known that n-hexane, methyl n-buty1 ketone, acrylamide, produce characteristic ultrastructural lesions: some axons are distended by proliferations of 9 to 10 nm diameter filaments. These features may be discovered by chance on a NB of a drug-abusing patient (for instance a glue-sniffer).

Toxic encephalopathies
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Toxin-induced injuries of the central nervous system (CNS) are an increasing problem due to the ubiquitous use of industrial products, the cost and difficulties in disposing its waste, the continued development of novel chemical compounds, and their growing use for therapeutic and recreational purposes. Exogenous toxins may be natural or synthetic, particularly drugs and include gases (eg. carbon monoxide), metals (such as mercury), liquids (ethanol, solvents) and many solids. Endogenous toxins include free radicals, glutamate, paradoxically an excitatory neurotransmitter, when its concentration reaches a level to produce neuronal apoptosis. Whether ingested, inhaled, or absorbed through the skin, symptoms may appear immediately or be delayed, are non-specific and may include loss of memory, vision, and/or intellect, headache, cognitive and behavioral changes, seizures, narcosis, anesthesia, respiratory arrest, unconsciousness and coma, due to a broad range of effects such as selective neuronal damage and impaired neuronal-glial metabolism.

Neuropathological investigations are complex because of the intricate spatial and temporal diversity in the anatomic, functional, and molecular organization of the CNS. Substances with similar structural features or common mechanisms of action may produce divergent changes. Edema, the most striking macroscopic finding in acute encephalopathies, may be secondary to vascular damage, as in lead encephalopathy, or related to myelin damage, as with triethyl tin. When resulting from diffuse cytotoxic damage, as in thallium intoxication, affects both grey and white matter, giving a ‘moth eaten’ appearance to the cortex. Basal ganglia and the hippocampus are usually damaged in disturbances of energy supply. Histologically, mild edema, myelin pallor and gliosis may be observed. Most descriptions are in animal models, due to the low likelihood that cases will come to autopsy, or referred to specialist centers, but described data reflect end-stage disease, often difficult to place in the context of etiology and pathogenesis, there are several important theories including neural connectivity, neural migration, excitatory-inhibitory neural activity, dendritic morphology, neuro-inflammatory, calcium signaling and mirror neurons, which may offer an explanation of the development of ASD or at least aspects of the phenotype. In this workshop, you will hear about the neuropathology of ASD and experimental work in animal models and humans that have advanced our understanding of this complex neurodevelopmental disorder. In my short introduction, I shall focus on the genomic studies that have revealed that a substantial proportion of ASD risk resides in high-impact rare varia-

W12: Unraveling the neuropathology of autism
Introduction into autism genetics
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Autism spectrum disorder (ASD) is a complex developmental disorder with a seemingly confusing and uncertain pathogenesis. The definitive mechanisms that promote autism are poorly understood and mostly unknown, yet available theories do focus on the disruption of normal cerebral development. Although there is no clear pathway or simple pathogenesis, there are several important theories including neural connectivity, neural migration, excitatory-inhibitory neural activity, dendritic morphology, neuro-inflammatory, calcium signaling and mirror neurons, which may offer an explanation of the development of ASD or at least aspects of the phenotype. In this workshop, you will hear about the neuropathology of ASD and experimental work in animal models and humans that have advanced our understanding of this complex neurodevelopmental disorder. In my short introduction, I shall focus on the genomic studies that have revealed that a substantial proportion of ASD risk resides in high-impact rare varia-
Brain region specific alterations of neuronal number and volume in autism
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A total of 38 brain cytoarchitectonic subdivisions, representing subcortical and cortical structures, cerebellum, and brainstem, were examined in 4- to 60-year-old autistic and control subjects to detect (a) global patterns of developmental abnormalities and (b) to establish whether the function of developmentally modified structures correlates with the behavioral alterations that are diagnostic for autism. The volume of cytoarchitectonic subdivisions, neuronal numerical density, and total number of neurons per region of interest were determined in 14 autistic and 14 age-matched controls using unbiased stereological methods. The study revealed that significant differences are limited to only a few brain regions, including the cerebellum and some striatum and amygdala subdivisions. In the autistic group, the total number and numerical density of Purkinje cells in the cerebellum were reduced by 26% and 24%, respectively. In the amygdala, a significant reduction of neuronal density was limited to the lateral nucleus (by 12%). The another sign of the topographic selectivity of developmental alterations was an increase of the caudate nucleus and nucleus accumbens volumes by 22% and 34%, respectively, and reduced numerical density of neurons in the nucleus accumbens and putamen by 15% and 13%, respectively. The observed pattern is consistent with the results of magnetic resonance imaging studies and their clinical correlations, and some morphometric studies which indicate that structural defects of these three brain regions may contribute to social and communication deficits, and repetitive and stereotypical behaviors.

Cortical organization and synaptic culling in individuals with autism
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Autism spectrum disorders (ASD) are associated with multiple cortical alterations that implicate neurodevelopmental events during the prenatal and perinatal periods. There now exists numerous findings suggesting connectional changes within the cortex. One early and important cell population in this regard is the cortical preplate: a collection of neurons located at the zone that will later be occupied by migrating cortical neurons. Many preplate neurons persist into the adult brain as the cortical subplate, and they play a role in neuronal patterning within the cortex, and are responsible for guiding thalamocortical and cortico-thalamic connections. In addition, they provide early coordinated activation of the underlying cortical layers and, in the adult, may serve an additional role as modulators of cortical afferents. Although many subplate neurons undergo apoptosis during development, in primates a substantial proportion of these cells are retained into adulthood. ASD individuals demonstrate supernumerary neuronal profiles within the white matter directly adjacent to the overlying cortex. Initial findings indicate that many of these profiles show morphological characteristics of subplate neurons. Like in ASD, the presence of excess neurons adjacent to the cerebral cortex has been shown in other neurological disorders such as schizophrenia (Eastwood & Harrison, 2003) and epilepsy (Emery et al., 1997). In these conditions, it has been hypothesized that these neurons contribute to connectivity in the mature brain (Kostovic et al., 2011). As such, excessive neuronal profiles in the subplate may be a contributor to both abnormal cortical development and connectivity in the ASD brain.

Crossing the equator: Ube3a in Idic15 autism and Angelman syndrome
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Maternally derived increases of Ube3a gene dosage cause the autism-related behavioral deficits of reduced social and increased repetitive behaviors (maternal isodicentric chromosome 15 [Idic15] with neurolonal Ube3a triplication, Smith et al., Sci Transl Med 2011), whereas maternally derived Ube3a deletions cause Angelman syndrome characterized by severe intellectual and language disabilities, a movement disorder, epilepsy, and a pathognomonic increase of social interest, laughing and smiling (Jiang et al., Neuron 1999). Thus, Angelman syndrome and Idic15-associated autism and present with some diametrically opposite clinical features such as increased and decreased pro-social behaviors. Idic15 can also present with anti-social (aggression) behaviors. Ube3a is expressed exclusively from the maternal allele in mature neurons explaining the exclusive maternal inheritance pattern. Ube3a acts as an E3 ubiquitin ligase but also, independently, as a transcriptional co-activator. We are utilizing molecular genetic techniques to engineer the full-length Ube3a gene in transgenic mice in order to understand the cellular/molecular and brain circuit mechanisms where by Ube3a regulates these social behaviors.
How new neuropathologists have been recruited and trained worldwide
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In collaboration with neuropathology educators worldwide, we surveyed the mechanisms used for training neuropathologists. In general, recruitment is non-systematic relying on encouraging interested candidates through medical school and neuroscience research training environments. Six countries have structured neuropathology training systems that allow entry directly from medical school (i.e. a primary specialty). Others consider neuropathology a subspecialty, usually of anatomical pathology. The process for admission into the training programs varies. Some countries have a nationwide interview process and a centralized matching process (e.g. Canada, UK). Others (e.g. US) rely on site-specific recruitment, although the advantages of a matching system for undersubscribed postings are recognized. In jurisdictions with national oversight the training routes are remarkably similar. Most have a defined set of core competencies for diagnostic neuropathology overlaid with more general competencies required for any type of medical specialist. Eligibility for certifying examinations requires completion of a time-based training program that typically involves 3–4 years core neuropathology for the primary specialty or 2 years for the subspecialty. As a small discipline, one of the common difficulties for educators is teaching in the subspecialty areas of neuropathology. This is addressed through the offering of defined courses offered through national societies or supranational societies (e.g. EuroCNS). Some countries (e.g. Canada) are developing web-based lecture series to complement local training. One long-term challenge for educators will be to ensure that diagnostic neuropathology integrates rather than delegates evolving complementary methods such as genetic testing and advanced imaging and microscopy methods.

Young Investigation Franz Nissl Award
Neuropathologic subtypes of Alzheimer’s disease: a clinicopathologic and neuroimaging perspective
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Neurofibrillary tangle pathology in Alzheimer’s disease (AD) can be staged according to stereotypic involvement of entorhinal cortex and limbic regions, before involving association cortices and finally primary cortices (Braak & Braak 1991). At autopsy, however, ~25% of cases do not fit expected neurofibrillary tangle distribution according to Braak staging (Murray et al., 2011). Two atypical subtypes of AD were found based on the relative sparing of the hippocampus (i.e. hippocampal sparing [HpSp] AD) compared to the cortex, and the predominant involvement of hippocampus and amygdala (i.e. limbic predominant [LP] AD). Using thioflavin-S microscopy, we designed a mathematical algorithm to identify the atypical and typical AD cases in order to investigate clinicopathologic differences. HpSp AD and LP AD not only differed neuropathologically, but also showed distinct demographic and clinical patterns. Across each variable, HpSp AD and LP AD greatly contrasted with typical AD centered between the two. HpSp AD were more often male, younger at age onset, had shorter disease duration, and had an underrepresentation of the APOE ε4 genotype and MAPT H1H1 haplotype. Further studies validated differences using both immunohistochemistry and structural neuroimaging. Future studies will seek to utilize these AD subtypes as a specific phenotype to help inform both the genetics and atypical clinical presentations to inform the differential diagnosis of AD dementia.