Posters

Epilepsy

P01

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Microglia in cortical and subcortical autonomic brain regions in SUDEP

Introduction: Sudden unexpected death in epilepsy may arise as a result of autonomic dysfunction during a seizure. Evidence from quantitative MRI studies in SUDEP show volume changes in brain regions of the Central Autonomic Network (CAN) (Wandschneider, Koepp et al. 2015; Ogren, Tripathi et al. 2018) and functional MRI in patients at risk for SUDEP shows altered connectivity between CAN regions (Tang, Chen et al. 2014; Macey, Ogren et al. 2015; Allen, Harper et al. 2017). Neuroinflammation is both a cause and a consequence of seizures and can mediate neuronal dysfunction. Our aim was to evaluate microglial populations in CAN in SUDEP.
Methods: In 55 post-mortem (PM) cases (22 SUDEP, 17 epilepsy-controls and 16 non-epilepsy controls), Iba1 microglial marker was quantified using whole slide scanning/automated image analysis. 14 ROI were selected to include known CAN regions (pulvinar, thalamus, insular cortex, anterior cingulate) and comparison regions.
Results: We identified a significantly increased Iba1 labelling over all regions in SUDEP compared to epilepsy-controls ($P < 0.001$) and non-epilepsy controls ($P < 0.0001$). The most significant differences were noted in parahippocampal, temporal, cingulate, frontal cortex, thalamus and pulvinar, with lateralisation of some regions. Higher Iba1 labelling was present in both lesional and non-lesional SUDEP compared to epilepsy controls ($P < 0.00001$ to 0.05).
Conclusions: These features could indicate enhanced neuroinflammation in CAN in SUDEP. This may represent a marker of increased seizure activity but also a risk factor for SUDEP. Further work is required to investigate other markers of microglial activation.

P02

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Volumetric and neuropathological study of the medulla in SUDEP and correlates with 9.4T MRI

Background: Sudden unexpected death in epilepsy is likely a result of autonomic dysfunction during a seizure. MRI volume reduction in brainstem autonomic regions correlated with autonomic symptoms in epilepsy and SUDEP patients [1]. We have recently identified alterations in neuronal populations in the ventro lateral medulla (VLM) and medullary raphe (MR) in post mortem samples [2]. We now extend these studies to address volumetric changes and pathology in other medullary regions in SUDEP.
Methods: In 47 cases (18 SUDEP, 18 non-epilepsy controls (NEC) and 11 epilepsy controls (EC)) the following regions of interest were delineated: VLM, MR, solitary tract (ST), inferior olive (IO) and entire reticular formation (RF). The Cavalieri stereological method was used to measure both actual and relative volumes of ROI. Immunolabelling index for myelin (SMI94), neurones (MAP2) and gliosis (GFAP) was also measured in ROIs. In 16 cases with additional 9.4T MRI we also evaluated T1, T2, T2* and MTR to correlate with pathology measures.
Results: There was a trend for increased relative volume of the VLM in SUDEP compared to controls ($P < 0.05$) in the caudal medulla (obex < 6 mm) but no evidence for actual volume reduction in any ROI. There was a correlation between MRI and pathology measurements but there were no significant differences between the SUDEP and control groups for T1, T2, T2* and MTR in any ROI in either caudal or rostral medulla levels (obex < or >6 mm) .
Conclusions: There is limited evidence from post mortem volumetric studies to support significant volume alterations in medulla regions in SUDEP or differences in quantitative MRI measurements.

P03

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Medullary catecholaminergic neurones in sudden unexpected death in epilepsy

Introduction: Seizure-related autonomic dysfunction may underlie sudden unexpected death in epilepsy (SUDEP). In a previous study we showed a reduction in medullary raphe (MR) and serotonergic (tryptophan hydroxylase (TPH)) neurones in SUDEP [1]. Medullary catecholaminergic neurones regulate arterial BP and cardio-respiratory arousal in hypoxia. Tyrosine hydroxylase (TH) in the medulla identifies C1 neurones in ventro lateral medulla (VLM) and C2/C3 neurones in nucleus tractus solitarius (NTS).
Methods: Serial 20 μm sections through medulla from 18 SUDEP cases, 9 epilepsy controls (EC) and 18 non-epilepsy controls (NEC) (obex 0–12 mm) at were immunolabelled for TH at 200 μm intervals and double labelled for TH/TPH. The slides were digitised on Leica slide scanner and regions of interest (ROI) defined (VLM, MR, NTS); immunolabelling index and neuronal numbers were evaluated.
Results: Although higher TH labelling was present in SUDEP and EC in the VLM and NTS than NEC there were no significant differences. TH labelling between NTS and VLM was significantly different in NEC ($P < 0.05$) but not significant in either EC or SUDEP groups. Identification of TH neurones in MR is a novel finding and TPH/TH co-expression was observed in NEC. TH labelling correlated with TPH only in the MR and only in the NEC group ($P < 0.0001$).
Conclusions: There is no evidence for a reduction of TH catecholaminergic medullary neurones in SUDEP. Differences in the relative distribution in epilepsy groups to controls could suggest seizure-related alterations of C1/C2 neurones of potential relevance to cardio-respiratory modulation during seizures. Future work is required to address other brainstem catecholaminergic neurones and correlation with ictal cardiovascular/autonomic symptoms.

P04

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Adenosine kinase and Adenosine receptors A1 and A2A in temporal lobe epilepsy and association with risk factors for SUDEP

Introduction: The "adenosine hypothesis of SUDEP" predicts that a seizure-induced adenosine surge in combination with impaired metabolic clearance can trigger a lethal apnoea or cardiac arrest. Changes in adenosine receptor density have been observed in surgical epilepsy patients. Our aim was to study the distribution of adenosine kinase (ADK) and adenosine receptors (A2A and A1) in patients with temporal lobe epilepsy and hippocampal sclerosis (TLE/HS) and correlate this with risk factors for SUDEP.
Methods: In 75 cases, SUDEP-7 inventory pre-operatively categorised patients into high risk ($n = 16$), medium risk ($n = 11$) and low risk ($n = 48$) groups. Whole slide digital images were analysed using Definiens to quantify the labelling index (LI) for ADK, A2A and A1 LI in 7 regions of interest (ROI): temporal cortex, temporal lobe white matter, CA1, CA4, dentate gyrus, subiculum and amygdala. We also correlated ADK, A2A and A1 relative to glial (GFAP) and neuronal (NeuN) LI in these ROI.
Results: A2A showed mainly astroglial cytoplasmic expression, A1 mainly neuronal cytoplasmic expression and ADK nuclear labelling in mixed cell types in all ROI. Significantly lower A2A LI was shown in the temporal cortex in high risk compared to low risk SUDEP cases ($P < 0.05$) but no significant differences for other ROI or for ADK or A1. When expressed as a ratio of GFAP, this significance for A2A increased in the cortex ($P < 0.001$) as well white matter ($P < 0.05$).
Interpretation: A decrease in cortical astroglial A2A receptors in TLE/HS could implicate defective adenosine signalling in high risk for SUDEP patients. This may indicate an underlying astrogliopathy as a risk factor for SUDEP.

Cerebrovascular disease

P05

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Identification of miRNA and mRNA regulatory networks in the ageing blood-brain barrier: Comparative gene expression studies in human and mouse

Introduction: We have recently shown that blood brain barrier (BBB) dysfunction is a feature of brain ageing in both human cohorts and mouse models. The current study aims to identify mRNA and miRNA expression changes that contribute to age-associated microvascular pathology, including BBB dysfunction.
Methods: Collagen IV+ microvessels were isolated from the cortex of ageing-representative human and mouse cohorts using immuno-guided laser capture microdissection, and changes in mRNA determined using Affymetrix gene chips and miRNA assessment using Qiagen QPCR miRNome arrays. The datasets were analysed using the PUMA package from Bioconductor in RStudio to identify relevant age-associated mRNA and miRNA changes above 1.2 fold and with a *P*-value of less than 0.05.
Results: Seven candidate genes were selected and validation was carried out in the laboratory using immunohistochemistry and quantitative PCR. Immunohistochemistry revealed that three of the four genes tested were present in the endothelium of cerebral microvessels. Quantitative PCR showed that four of the six genes tested exhibited age-related expression changes consistent with the direction of changes seen in the microarray analysis.
Conclusion: This study provides a greater understanding of the mRNA/miRNA network changes that occur in ageing and may help to develop novel therapies for BBB dysfunction in neurodegenerative conditions.

Future work using deconvolution approaches will allow correlation of pathway changes to particular cell types, and network analysis studies will be used to identify relevant pathway changes with ageing.

P06

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The effect of systemic atherosclerosis in the neurovascular unit

Background: Atherosclerosis is a chronic disease affecting major blood vessels, including those that supply the brain. Cerebral microvascular dysfunction is implicated in the pathogenesis of dementia, but how systemic vascular disease affects the microvasculature within the central nervous system is currently unknown. We hypothesise that systemic atherosclerosis is associated with changes in the brain microvasculature, including glial responses that could lead to dysfunction of the neurovascular unit (NVU) and contribute to neurodegeneration.
Methods: Hippocampus, thalamus, basal ganglia, corpus callosum and cerebral cortex regions were sampled from an Apolipoprotein E knockout (ApoE-/-) mouse model of atherosclerosis in animals fed on a high fat diet ($n = 7$) or low fat diet ($n = 7$). Astrocyte (GFAP) and microglial (Iba-1) pathology were assessed using a standard immunohistochemistry approach, and the percentage area immunoreactivity in the regions of interest assessed by image analysis.
Results: Iba-1+ microglia were a prominent feature of all brain regions from animals fed on a high fat diet, with significantly higher levels of % area immunoreactivity detected in the hippocampus ($P = 0.0042$), thalamus ($P = 0.0012$), basal ganglia ($P = 0.0009$), corpus callosum ($P = 0.0023$) and cerebral cortex ($P = 0.0045$). GFAP+ astrocytes were detected in all brain regions of animals fed on either a low fat or high fat diet.
Conclusions: The neuroinflammatory response to atherosclerosis indicates that systemic atherosclerosis is associated with changes in the cerebral microvasculature, affecting astrocyte and microglial responses that could contribute to neurodegeneration. Future research will expand the histological characterisation studies, specifically assessing the detailed phenotype of the microglial response.

P07

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Carotid artery disease, strokes and experimental effects of enriched environment on stroke injury

Background: Carotid artery disease (CAD) is an important risk factor for stroke injury. However, it is not clear how much cross-talk there is between extracranial large artery disease and intracranial small vessel disease.
Methods: A total of 70 post-stroke cases from the Cognitive Function After Stroke (CogFAST) study were assessed the type and extent of stroke and carotid artery pathology. We then similarly quantified stroke pathology as a consequence of carotid artery stenosis in a mouse model of bilateral common carotid artery stenosis (BCAS). We explored effects of two different paradigms of limited and full-time exposure to Enriched Environment (EE) on subsequent stroke injury and cognitive function after BCAS.
Results: In the human cohort, stroke survivors with severe CAD (>75% area stenosis) developed greater numbers of cortical >subcortical small infarcts. Severity of carotid artery stenosis was associated with greater risk of developing dementia. In the experimental mouse cohort, BCAS reduced cerebral blood flow by 52% compared to sham animals ($P < 0.01$). BCAS also induced stroke pathologies, and total and cortical infarct volumes were reduced by ~50% in BCAS plus limited and full-time EE compared with BCAS without EE ($P < 0.01$). We further demonstrated frontal cortical stroke volumes linked to working memory deficit. Proteomic analysis revealed that EE lead to attenuation of coagulation cascade factors in brains of BCAS compared to BCAS without EE.
Conclusions: While our results show that CAD has a role in small infarcts, experimental evidence strongly suggests that EE significantly reduces subsequent stroke injury. EE appears a safe and effective interventional strategy for patients with CAD and strokes.

P08

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Adrenergic receptors in the walls of cerebral vessels as possible targets for improving intramural peri-arterial drainage in CAA

The deposition of amyloid-β in cerebral amyloid angiopathy (CAA) is due to a failure of clearance of fluid and proteins along Intramural Peri-Arterial Drainage (IPAD) pathways in basement membranes of cerebral capillaries and arteries. Improving IPAD could be a strategy for prevention and treatment of CAA and Alzheimer's disease (AD), but therapeutic targets for IPAD have been difficult to identify. Preliminary results suggest that Prazosin, an α1 adrenergic antagonist, reduces CAA in a transgenic mouse model, suggesting that adrenergic receptors could represent targets for improving IPAD. In this study, we seek to demonstrate the exact distribution of α1A and α2A-adrenergic receptors in the walls of blood vessels of human brains and in cell cultures. We used immunofluorescence and confocal microscopy on occipital tissue from old non-demented brains from Newcastle Brain Tissue Resource and demonstrated that α1A as well as α2A adrenergic receptors are associated with arterial smooth muscle cells in the walls of arteries and with the endothelia of capillaries. In a separate experiment, human astrocytes and cerebral endothelial cells (hCMEC/D3 cell line) were seeded on to coated glass coverslips and left to grow for 72 hours before being fixed with 4% PFA, immunostained for α1A and α2A adrenergic receptors and counterstained with DAPI. Both adrenergic receptors were expressed on human endothelial cells with weak staining on astrocytes. Future work will seek to identify the changes in distribution of adrenergic receptors on blood vessel walls that occur with age and AD as well as the pattern of immunostaining on human vascular smooth muscle cells.

P09

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Changes in the intramural peri-arterial drainage (IPAD) pathways after traumatic brain injury

Traumatic brain injury (TBI) is associated with deposition of proteins such as tau and amyloid in the brain parenchyma and in the walls of arteries as cerebral amyloid angiopathy (CAA). We have previously demonstrated that soluble peptides are eliminated from the brain along the walls of cerebral capillaries and arteries as Intramural Peri-Arterial Drainage (IPAD) and that this drainage fails when the structure of extracellular matrix in the walls of arteries changes with age and possession of Apolipoprotein E ε4 genotype. In this study we test the hypothesis that there are progressive changes in the composition of the extracellular matrix in a mouse model of TBI. Young adult mice were subjected to controlled cortical impact injury and perfusion fixation after 7 days or 28 days. Imunocytochemistry for basement membrane proteins, laminin and perlecan, followed by confocal microscopy and statistical analysis using paired samples t-test were performed. Results demonstrate a trend towards an increase in the percentage area of blood vessel walls immunostained for both perlecan and laminin. This work suggests that TBI results in a change of the composition of the vascular extracellular matrix similar to that seen in ageing. This change may create an environment in which proteins are more prone to aggregation and fibrillization and thus impede their elimination via IPAD pathways. Future work will include a larger sample size and testing IPAD after TBI.

P10

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Iba-1-/CD68+ microglia are a prominent feature of age-associated deep subcortical white matter lesions

Deep subcortical lesions (DSCL) are present in ~60% of the ageing population, and are linked to cognitive decline and depression. DSCL are associated with demyelination, blood brain barrier (BBB) dysfunction, and microgliosis. Microglia are the main immune cell of the brain. Under physiological conditions microglia have a ramified morphology, and react to pathology with a change to a more rounded morphology as well as showing protein expression alterations.

We assessed markers of microglia and vascular integrity in DSCL and radiologically 'normal-appearing' white matter (NAWM).

The Cognitive Function and Ageing Study (CFAS) provided control white matter (WM), NAWM and DSCL human post mortem tissue for immunohistochemistry using microglial markers (Iba-1, CD68 and MHCII), a vascular basement membrane marker (collagen IV) and markers of BBB integrity (fibrinogen and aquaporin 4).

The immunoreactive profile of CD68 increased in a stepwise manner from control WM to NAWM to DSCL. This correlated with a shift from small, ramified cells, to larger, more rounded microglia. While there was greater Iba-1 immunoreactivity in NAWM compared to controls, in DSCL, Iba-1 levels were reduced to control levels. A prominent feature of these DSCL was a population of CD68+/ Iba-1- microglia. There were increases in collagen IV, but no change in BBB integrity. Overall the study shows significant differences in the immunoreactive profile of microglial markers. Whether this is a cause or effect of lesion development remains to be elucidated.

Furthermore, this study demonstrates that Iba-1 is not a pan-microglial marker: a combination of markers is required to fully characterise the microglial phenotype.

Developmental neuropathology and muscle disease

P11

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Microglial dynamics in the developing and early postnatal human brain

The regional heterogeneity of microglia is well-documented in the adult human and rodent brains. Microglia proliferate in the adult at a rate of 0.08–2% in humans and 0.5–0.7% in rodents. They appear by the 4th gestational week (gw) in the telencephalon and are thought to colonise the brain by 24 gw. Their precise spatiotemporal dynamics during development and in postnatal life remain unclear. With ethical approval from the above centres, frontal and temporal areas in 92 human control cases are currently being studied (age range 5 gw to 18 years). Clustering of microglial signature genes in RNA-seq data from 322 tissues was tested by region of interest and timepoint (5–22 gw). Preliminary results from the 25 gw to 2 years age range show very little microglial proliferation during the third trimester. Microglial morphology becomes more consistent with the mature adult form in the grey and white matters after 35 gw. Microglial proliferation increases dramatically soon after birth and by about 5 postnatal weeks, proliferation decreases sharply. RNA-seq data demonstrate clustering of microglial signature genes from the 5th gw in the cerebral cortex, the choroid plexus, the cerebellum and the spinal cord. These findings suggest that microglial proliferation is a postnatal event and that microglial signature genes cluster early by anatomical regions known to have differential microglial profiles in the adult. This work is part of a larger study investigating microglial dynamics across the lifespan in humans from the 4th gestational week until old age.

P12

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A versatile, modular digital script for automated high-throughput multiparametric myofibre analysis in brightfield and epifluorescent paradigms

Digital scripts are vital for unbiased, high-throughput multiparametric analysis of muscle landscapes in frozen/fixed histology sections. We have developed a series of image analysis methods with Definiens software, applicable to digital scans of chromogenic/fluorescent stained entire sections of skeletal muscle. Initially the script was developed for global landscape assessment, resolution then increased by achieving fibre separation, followed by multiplexed staining to investigate subpopulations of fibres. The global analysis mapped oxidative changes in the mixed fibre-type gastrocnemius muscle by measuring COX-SDH staining intensity translated to digital heat-maps in a long-term rodent model of critical illness and recovery. Initial analysis at the single fibre level was problematic due to lack of boundary definition, highlighting the importance of using an ubiquitiously expressed membrane marker as a sarcolemma-defining mask, introduced in subsequent analyses. Brightfield analysis of muscle fibre diameter in 'histologically normal' paediatric muscle biopsies provided good correlation between whole section counts and manually selected transverse regions, providing age-stratified data on muscle fibre size. A key feature of the script is background normalisation for maximising fluorescent signal: noise ratio. Techniques used include a moving average method and thresholding based on a global average of background signal. This technique was applied to quantify dystrophin expression in transverse sections from Duchenne muscular dystrophy biopsies. Further script development relates to the analysis of additional markers in sections with three or more multiplexed stains. In conclusion, our unique modular approach allows for continuous machine learning, increasing the script's capacity to generate a

variety of high-throughput qualitative and quantitative datasets with a wide range of neuromuscular applications.

P13

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Recessive loss-of-function mutations in ITGA7 cause cardiac arrhythmia with or without structural cardiomyopathy and respiratory muscle weakness

Integrin α7 encoded by ITGA7 is highly expressed in skeletal and cardiac muscle and contributes to sarcolemmal stability by binding to laminin α2. Three unrelated patients were previously reported with ITGA7-linked congenital muscular dystrophy. Here, we report three patients from two unrelated families presenting with adult-onset cardiac arrhythmia and respiratory weakness due to recessive null mutations in ITGA7. Patient I, a 51-year-old male presented with delayed motor milestones, stridor since birth and cardiac arrhythmia requiring ICD at 46 years. Cardiac MRI showed basal septal hypertrophy and fibrosis. Examination showed focal wasting of medial gastrocnemius and respiratory impairment. EMG was myopathic. Two other male siblings also reported cardiac symptoms. Patient IIa, a 61-year-old female presented at 49 years with episodic relapsing respiratory insufficiency requiring mechanical ventilation and tracheostomy. She had a history of atrial flutter, LBBB and AV-block requiring a pacemaker. Examination showed mild ankle dorsiflexor weakness and vocal cord paresis. EMG was myopathic. Muscle ultrasound was abnormal. Patient IIb, her female sibling aged 55 presented with chronic hypoventilation. She had mild limb weakness. Presently she uses nocturnal non-invasive ventilation. Quadriceps biopsies (PI and PIIa) revealed nonspecific myopathic changes. Next generation sequencing revealed a homozygous c.806_818del [p.S269fs] variant (PI) and two canonical splice site variants (PIIa, PIIb), (c.2357+1G>A [r.spl?] and c.2278-1G>A [r.spl?]) in ITGA7. Immunostaining revealed absent sarcolemmal integrin α7 labeling in both biopsies. Evaluation of α7-integrin-null mice showed a mild progressive myopathy with mainly diaphragmatic involvement and similar pathological features. Patients with predominant respiratory weakness and/or cardiac arrhythmias with or without structural cardiomyopathy should be screened for mutations in ITGA7.

P14

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Congenital fatal cap-rod myopathy due to a de novo autosomal dominant pathogenic ACTA1 variant

Cap disease is a rare structural congenital myopathy (CM) associated with hypotonia, proximal and facial muscle weakness, and frequently scoliosis and respiratory involvement. Mutations in TPM2, TPM3 and ACTA1 have been associated with cap disease, as well as nemaline myopathy. Combined caps and nemaline rods have been reported in the same patient due to a mutation in TPM3. Here, we report the first case of a severe, fatal CM with caps and nemaline rods. The patient was born at 37 weeks of gestation, with a history of polyhydramnios, little spontaneous movements at birth, generalised hypotonia, and required immediate ventilatory support. He died 20 days after birth. Ante mortem/post mortem biopsies from the quadriceps, biceps and diaphragm showed only diffuse fibre hypotrophy on light microscopy. Ultrastructural examination of the ante mortem quadriceps biopsy showed

several classical and atypical cap lesions. The post mortem quadriceps sample showed nemaline rods. Both samples showed capillary endothelial mitochondrial paracrystalline inclusions. Ultrastructural findings were key in directing molecular genetic testing. A next generation sequencing panel identified a de novo ACTA1 c.739G>C p. (Gly247Arg) variant previously reported in the literature in a patient with severe nemaline myopathy, affecting a highly conserved amino acid, and predicted to affect actin function with In Silico analysis. Our case of ACTA1-related cap-rod myopathy is the most severe presentation of a CM with caps or cap-rods described till date. The case further cements the notion of caps and rods being part of the 'nemaline spectrum' and highlights the remarkable heterogeneity of lesions within the same muscle or same group of muscles.

P15

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Extended phenotypic spectrum of VCP inclusion body myopathy: report of two cases with atypical early and late childhood-onset disease

Autosomal-dominant inclusion body myopathy with Paget disease of bone and frontotemporal dementia (IBMPFD) is a late-onset multisystem disorder due to mutations in the VCP (valosin-containing protein) gene. Myopathy is the commonest feature affecting 80–90% individuals with limb-girdle, scapulohumeral and distal-predominant patterns evolving to affect respiratory muscles and the heart. The most consistent pathological findings are VCP/ubiquitin/TDP43-positive intranuclear/cytoplasmic inclusions, rimmed vacuoles and tubulofilamentous inclusions. The inclusions are not IBMPFD-specific and are reported in other neurodegenerative disorders. Paediatric VCP-disease is hitherto unreported. Here we describe two clinically diverse early and late-childhood onset cases with unifying pathology of VCP inclusion body myopathy. PI, a 21-year-old male presented at age 16 with aching pain in his left forearm and progressive difficulty straightening the left hand fingers. Remaining neurological assessment was normal. CK was normal, EMG myopathic in left forearm finger flexors and intrinsic hand muscles, and muscle MRI showed oedema and fatty infiltration affecting left forearm and finger flexors. PII, a 22-year old female presented at age 9 with toe walking and delayed motor milestones. She developed rapidly progressive weakness and lost ambulation at age 14. She is currently fully wheelchair dependent, and uses long-term non-invasive ventilation. Muscle biopsies from both patients showed identical 'full house' pathology of VCP myopathy with chronic myopathic/dystrophic changes, rimmed vacuoles, sarcoplasmic and intranuclear protein aggregates/inclusions (VCP/ubiquitin/TDP43+) and tubulofilamentous inclusions. Extensive molecular genetic studies till date are negative including whole exome sequencing in PII, suggesting further molecular genetic diversity may underpin the VCP inclusion body pathology in these atypical paediatric presentations. Recruitment to other next-generation-sequencing platforms is under consideration.

P16

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Diagnostic challenges in paediatric anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase necrotising myopathy (anti-HMGCR-NM)

Paediatric inflammatory myopathies are rare and diagnostically challenging conditions, often mimicking inherited myopathies and dystrophies. Here we present the case of a 17 year old girl with anti-HMGCR-NM. The patient presented at age 9 years, with axial and proximal muscle weakness and bilateral inflammatory rash on forearms. CK was 12,000 IU/L. Immunosuppressive treatment, first with Prednisolone and Methothrexate, and later with Infliximab gave limited benefits and muscle weakness gradually worsened. Over 8 years, she developed joint contractures, as well as wasting in limb muscles. Cardiac function remained normal. In view of the axial and limb girdle weakness, muscle wasting, contractures and unresponsiveness to treatment, molecular investigations for limb girdle muscular dystrophies (LGMD), particularly LGMD1B were initiated, revealing a LMNA gene variant (R644C) of unclear significance, also found in healthy individuals. Muscle MRI of the lower limbs, at age 13 and 17 years, showed marked progressive, selective, symmetrical fatty atrophy of gluteal and thigh muscles, not in keeping with LGMD1B. Muscle biopsies at age 13 and 17 years favoured a necrotising myopathy on a background of chronic myopathic changes, and striking diffuse sarcolemmal complement C5b-9 deposits in the second biopsy. The differential diagnosis included inflammatory laminopathy. The patient was found positive for anti-HMGCR antibodies confirming the diagnosis of anti-HMGCR-NM. Careful review of the clinical history, repeated muscle MRI and muscle biopsy were key to confirm the correct diagnosis of anti-HMGCR-NM in this patient. This case highlights the diagnostic challenges for children with this rare condition, particularly in cases refractory to immunosuppression, and the importance of a multidisciplinary approach to diagnosis.

P17

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A novel case of MSTO1-related congenital muscular dystrophy with cerebellar involvement

Recessive mutations in the MSTO1 gene, encoding for a mitochondrial distribution and morphology regulator, have been recently described in four families with multisystem involvement, mostly characterised by myopathy or dystrophy, cerebellar ataxia, pigmentary retinopathy and raised CK. Here we report a patient with recessive MSTO1 gene related muscular dystrophy and ataxia. The patient, born to non-consanguineous parents, presented at age 2 years with global developmental delay. At age 15 years he was ambulant and showed axial, upper and lower limb weakness pronounced proximally, scoliosis, ankle contractures and ataxia. There was no cardiorespiratory involvement. EMG was normal. Brain MRI at 6 years showed cerebellar atrophy and mild under-opercularisation of the left Sylvian fissure; when repeated at 9 years, there was mild progression of cerebellar atrophy and additional supratentorial sulcal prominence suggestive of volume loss. Muscle MRI showed increased T1 signal in the lower limbs with normal STIR sequences. CK was raised (800–1614 IU/L). Vastus lateralis biopsy showed chronic dystrophic changes, few non-rimmed vacuoles and markedly reduced MSTO1 immunolabeling. Respiratory chain enzyme studies were normal.

Whole-exome sequencing revealed 2 missense MSTO1 variants. The first variant (c.766C > T p.(Arg256Trp)), affecting a conserved residue in the tubulin domain of the protein, is reported in the gnomAD dataset with an allelic frequency of 0.00003, while the second (c.1435C > T p. (Pro479Ser)) is novel. In silico tools predict both variants as damaging. Phasing of the variants is in progress. This case confirms a consistent phenotype associated with recessive MSTO1 gene mutations and suggests that progressive cerebellar atrophy can be a feature of the condition.

P18

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Prevalence of cytoplasmic bodies in a large series of diagnostic paediatric muscle biopsies

Cytoplasmic bodies (CB) in skeletal muscle biopsies typically appear as discrete, small sarcoplasmic inclusions that are eosinophilic and stain red with Gomori Trichrome (GT). The first description of CB as structural Z-disc anomalies was in 1969, and their association with desmin-related neuromuscular diseases (NMD) was recognised in the 1980s. Since then CB have been reported in association with a range of unrelated neuromuscular disorders, many of these in the pre-molecular era. The aim of our study was to look at the prevalence of CB in paediatric-onset NMD (0–16 years) and any particular genotypic correlation. A natural language search on the pathology database revealed documentation of CB in 41/1000 biopsies (0.04%) referred to our centre (2008–2017). Based on the tinctorial stains (Haematoxylin and Eosin (HE)/GT), frequency of CB was graded semiquantitatively (0, sparse:1+, <5 fibres: 2+, >5 fibres:3+, >10 fibres: 4+). The 41 cases with CB featured a variety of pathological diagnoses: centronuclear myopathy with/without cores (4/41), myofibrillar/protein aggregation myopathy(6/41), muscular dystrophy(5/41), nemaline myopathy(8/41), type II atrophy(2/41), neurogenic or mixed neurogenic-myopathic(2/41), mitochondrial myopathy(1/41), non-specific myopathy(12/41) and minimal change(1/41). CB were confirmed ultrastructurally in 5/21 cases, with similar light microscopic morphology of CB in cases with and without ultrastructural confirmation. CB were more frequent (3+) in the centronuclear myopathy group and a proportion of nemaline (3/8) and myofibrillar/protein aggregation (4/6) myopathies. 18/41 cases had a genetic diagnosis (BAG3, FHL1,CFL2,KHL40,LMOD3,NEB,MYH2,MYH7,RYR1, TTN, STAC3,RAPSYN,DMD,LMNA, and a case translocation t(9;11)). In conclusion, CB are a rare finding in paediatric muscle biopsies. They do not provide specific clues for an underlying gene defect and are probably non-specific indicators of myofibrillar modification.

P19

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SLONM: sporadic late-onset nemaline myopathy. A case study and review of neuropathological findings

Sporadic late-onset nemaline myopathy (SLONM) is a rare acquired adult onset myopathy characterised by progressive proximal limb and axial muscle weakness and the presence of nemaline rods in muscle fibres. In a significant proportion of cases, SLONM is associated with monoclonal gammopathy of unknown significance (MGUS), which is associated with an unfavourable outcome due to respiratory failure.

Histopathological features on muscle biopsy include atrophic and lobulated muscle fibres and, more specifically, frequent small 'sand-like' nemaline rods sometimes filling entire atrophic fibres. Nemaline rods are identified through modified Gomori trichrome staining and are also labelled by immunostaining for the Z-band protein myotilin. Further confirmation may involve electron microscopy, which shows the rods have a high electron density and an internal lattice structure.

We describe a case of SLONM-MGUS that presented with progressive proximal muscle weakness, mild neck stiffness, and loss of muscle bulk particularly in the quadriceps. Diagnosis was made on histopathological assessment of two muscle biopsies and with confirmation on electron microscopy.

The patient had a good outcome with autologous stem cell transplantation following high-dose melphalan and has been followed up for the past year.

P20

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Investigating senescence activation in response to oxidative DNA damage in neurones in vitro

Introduction: Cellular senescence and a senescence-associated secretory phenotype (SASP) have been described in mitotic cells but their role in post-mitotic cells such as neurones is not understood. We aimed to determine whether oxidative stress induces a persistent DNA damage response (DDR) and activation of senescence in human neurones in vitro.
Methods: Post-mitotic Lund human mesencephalic stem (LUHMES) cells were stressed with hydrogen peroxide to induce acute (ADD) and persistent (PDD) DNA damage. Changes in the transcriptome of ADD and PDD LUHMES were assessed using microarray analysis. GFP-LUHMES were co-cultured with ADD and PDD LUHMES or incubated with their conditioned media to investigate the development of a SASP; neurite outgrowth impairment and double-strand break (DSBs) formation were evaluated. Activity of senescence-associated β-galactosidase (SA-β-gal) was also assessed.
Results: Dysregulation of cell cycle, ATR and oxidative phosphorylation pathways was seen in PDD LUHMES. qRT-PCR and functional validation confirmed altered mitochondrial complex I but not cell cycle re-activation. A significant neurite growth impairment was seen in GFP-LUHMES co-cultured with PDD LUHMES ($P \leq 0.0001$), but co-culture conditions did not induce DSBs formation in GFP-LUHMES. SA-β-gal activity was present in control, ADD and PDD LUHMES and was not consistent with previous reports.
Conclusions: "Classical" senescence genes or pathways were not dysregulated in PDD LUHMES; however, DDR signalling, cell cycle regulation and oxidative phosphorylation were affected and could be linked to a senescent-like phenotype. PDD LUHMES had a detrimental effect over healthy GFP-LUHMES but not by directly inducing DNA damage. Finally, SA-β-gal activity might be affected by conditions different to senescence activation and should be interpreted with caution.

Brain tumours

P21

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Advanced molecular characterization using digital spatial profiling of immuno-oncology target expression in methylated versus unmethylated IDH-wildtype glioblastoma

Glioblastoma (GBM) is the most common primary adult brain tumor with a median overall survival of 12–15 months. Detailed molecular characterization of potential immuno-oncology biomarkers in GBM is required to predict the potential efficacy of novel immunotherapeutic agents.
Methods: We used Digital Spatial Profiling (DSP) to analyze 28 immuno-oncology proteins (PD1, PD-L1, B7-H3/CD276, VISTA, CD45, CD45RO, CD3, MS4A1/CD20, CD4, CD8A, CD68, GZMB Beta-2-microglobulin, CD56, Beta-catenin, FOXP3, Histone H3, CD14, CD19, AKT, P-AKT,Bcl-2, CD44, S6, IgG Rabbit Isotype Control, Mouse IgG Control, Pan-Cytokeratin, Ki67) conjugated to indexing DNA oligos with a UV photocleavable linker.

Multiple regions of interest (ROI) in formalin-fixed, paraffin-embedded tissue from 10 IDH-wildtype GBM cases (5 methylated and 5 unmethylated) were selected with fluorescently labelled antibodies, and oligos were released via UV mediated linker cleavage. Free oligos were captured via microcapillary fluidics into a microtiter plate and then quantitated. An nCounter platform allowed quantitative comparisons of antibodies between ROIs in MGMT methylated and unmethylated tumours. Mean protein expression levels between methylated

and unmethylated samples were compared using a linear mixed effect model.

Results: DSP shows 10 immuno-oncology target proteins (CD4,CD14, CD68, CD8A, B7.H3, PD.L1, CD19, FoxP3, CD44 and STAT3) were significantly increased in methylated versus unmethylated IDH wild-type GBM (after controlling the false discovery rate FDR adjusted P value <0.1 by Benjamini-Hochberg Procedure).

There was no relation between individual protein expression and overall survival.

Conclusions: Our results show increased immuno-oncology target expression in methylated versus unmethylated IDH wildtype GBM. Advanced immune-oncological biomarker analysis is required to identify predictive biomarkers for novel immunotherapeutic agents in GBMs.

P22

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Modelling high-risk paediatric brain tumour infiltration

Introduction: Malignant paediatric brain tumours are often difficult to diagnose, demonstrate clinically aggressive behaviour, and a poor prognosis. The infiltrative, diffuse nature of many of these tumour entities, including medulloblastomas and high-grade gliomas, can render treatment strategies ineffective. The ability to model these intrusive patterns microscopically will lead to a better understanding of both their mode of infiltration and surrounding microenvironmental interactions.

Methods: Brain tumour tissue resected during surgery was dissociated into single cell suspensions and grown as either 2D adherent cultures or 3D neurospheres. Cells were injected into orthotopic slice cultures and grown. Tissue clearing and subsequent immunostaining of key lineage markers were performed.

Results: Primary patient-derived cells were successfully injected into in vitro brain slice cultures and grown for several days. Visualisation by immunohistochemistry enabled the determination of both patterns of infiltration and tumour cell-cell interactions to be analysed.

Conclusion: These ex vivo paediatric brain tumour models can now be used to functionally test the hypothesized underlying molecular mechanisms that promote tumour infiltration. This modelling technique facilitates specific tumour type exploration of these rare tumours, prior to the use of preclinical in vivo animal models.

P23

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DNA methylation profiling in paediatric CNS tumours

Introduction: The performance of methylation classification according to the DKFZ model has not been fully assessed in a paediatric setting. Presented here is the impact of array inclusion on the final diagnosis of paediatric cases (≤21 years) reported during a 24-month period.

Materials and Methods: DNA was extracted, bisulphite converted and restored before processing on Illumina MethylationEPIC arrays. Methylation data was analysed using the DKFZ model and outputs recorded. We evaluated the impact of array inclusion on the final reported diagnosis, and assessed the concordance of copy number data in relation to other molecular findings.

Results: As part of our routine clinical service, we performed methylation arrays on a total of 311 cases; estimated to represent 60% of all CNS tumours seen locally. Robust classification was achieved in half of all paediatric cases tested, and this data positively contributed towards the final diagnosis in the majority of these. Gene amplification assessed by FISH mostly correlated with inferred copy number plots and also contributed towards the final diagnosis.

Conclusions: DNA methylation arrays and the classifier model are valuable adjuncts to paediatric neuropathology and a fully integrated diagnosis of CNS tumours.

P24

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Characterisation of histone mutant gliomas in adults at QEHB 2015–2018

Introduction: Neuropathologists become increasingly aware of gliomas harbouring mutations in histone H3F3 A/ HIST1H3B/C, associated with young age and poor outcome. Histone mutations were initially recognised in paediatric high grade gliomas, but are increasingly identified in adult gliomas. We reviewed epidemiology, radiology, neuropathology, molecular profile and clinical outcome in our practice at UHB during 2015–18 and correlate the findings with the current literature.
Results: Among 35 gliomas analysed, 9 (26%) histone mutant gliomas were identified: 5 cases with H3 K27M mutation, leading to a diagnosis of diffuse midline glioma (DMG), WHO grade IV, and 4 cases with H3 G34R mutation regarded as subtype of hemispheric glioblastoma (GB). We confirm that the age spectrum of DMG is wide, ranging from 18 to 43 in our cohort, in contrast to patients with H3 G34-mutant glioma restricted to 17–19 years.

DMG histology included various phenotypes such as ependymoma, anaplastic astrocytoma and GB, while morphology of G34R mutants comprised anaplastic ganglioglioma, GB with primitive neuronal component and conventional GB. Only one DMG was low grade (10% low grade reported in the literature). All tumours were IDH-wildtype. While the majority (80%) of K27M mutants had ATRX wildtype and variable p53 status, all (100%) G34R mutants carried ATRX and p53 mutations. While most DMGs had unmethylated MGMT, interestingly, all G34R mutants showed very high MGMT methylation (46–70%). 3/9 patients (33%) died within 2 years from diagnosis.
Conclusion: Histone mutant gliomas have a wide neuropathological spectrum and occur in adults, especially DMG. Therefore, testing for histone mutations should be considered in all adult IDH-wildtype gliomas.

P25

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EGFR as a potential prognostic biomarker in adult IDH-wildtype glioblastomas

Introduction: EGFR amplification and EGFRvIII mutation are the most common EGFR alterations in glioblastomas, but their prognostic value is still unsettled. This study aims to evaluate the EGFR prognostic value in adult IDH-wild-type glioblastomas, in four glioblastoma variants (glioblastoma, glioblastoma-with-sarcomatous-elements, giant-cell-variant, small-cell-variant).
Methods: This study included 126 primary glioblastomas specimens from patients over 30 years old (KCH, 2012–2017). Reverse transcription–polymerase chain reaction was performed for EGFRvIII analysis and fluorescence in situ hybridization for EGFR amplification testing. IDH mutation status was confirmed by pyrosequencing and the MGMT promoter methylation status had been previously evaluated.
Results: From the 109 cases studied for EGFR amplification (EGFR copy numbers ≥15 in 10% or more tumour cells) 38 presented amplification and 26 of these were also EGFRvIII mutated. Both methylated (median overall survival = 22.8 months) and EGFR amplified (median overall survival = 21.6 months) patients presented better overall survival comparing to unmethylated (median overall survival = 10.8 months) and non-amplified glioblastomas cases (median overall survival = 9.6 months), respectively ($P = 0.002$; $P = 0.007$).
Discussion: EGFR amplified glioblastomas were correlated with a better overall survival independently of MGMT promoter methylation status, suggesting its value as a prognostic factor within our clinical setting. The same association was not found with EGFRvIII mutation. Although no particular difference in the molecular profile between the four subtypes was found, when analysed together with other factors such as age, MGMT promoter methylation status, EGFR amplification and Ki-67 index, glioblastoma giant-cell-variant can potentially suggest a better overall survival and potential be considered on patient's management.

P26

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A rare case of fibroblastic reticular cell tumour in the spine

Introduction: Dendritic/reticular cells are diveded into 3 major subsets: follicular dendritic cells, interdigitating dendritic cells and fibroblastic reticular cells (FRC). FRCs are stromal support cells located in the parafollicular area and deep cortex of lymph nodes. FRCs have myofibroblastic-like features, in that they are immunohistochemically reactive for vimentin, smooth muscle actin and desmin, and negative for CD21, CD35 and S-100 protein. Most of the reported tumours derived from these cells represent FDC sarcomas, and only 21 cases of FRC tumours have been reported. Hereby we present a case of FRC tumour in the spine.
Case report: A 45-year-old female patient presented with 2 weeks of bilateral ascending paraesthesia and unsteadiness. MRI revealed a T3/T4 extradural lesion with central enhancement, suggesting lymphoma. The tumour was surgically debulked and histology showed blunt spindle cells with slightly oval nuclei admixed with small numbers of lymphocytes and plasma cells. Fine reticulin fibres surrounded many of the tumour cells with no packeted arrangement. Mitotic figures were absent. A panel of immunohistochemistry excluded common type of lymphomas and CD45, S100 protein, fascin and SMA were variable positive. CD21, CD23 and CD35 were negative. This immunophenotype excluded follicular dendritic cell sarcoma and interdigitating dendritic cell sarcoma. The diagnosis of fibroblastic reticular cell tumour (FRCT) was made.
Conclusions: FRCT is very rare and probably underrecognised. The clinical outcome is variable, their behaviour has been more in keeping with that of low-grade sarcomas than with that of malignant lymphomas, being characterized by local recurrences and occasional blood-borne metastases.

P27

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Audit of turnaround time (TAT) in glioma diagnosis

'Glioma' is a tumour of the neuroglia of the central nervous system. Since July 2016, integrated glioma diagnoses are formed between molecular genetics and standard histological techniques. Turnaround time (TAT) between surgery and initial (histological) diagnosis, and TAT between initial and integrated diagnoses, are important to consider. TAT should be minimised: the goal for initial TAT is 7 working days. Integrated TAT has no guideline, but the same time can be aimed for. The aim of this audit is to assess initial and integrated TAT in SJUH, consider changes to diagnoses after genetic testing, and make suggestions for improvement. 45 patient reports were analysed. 36 reports were complete – most achieved the target initial TAT (median 6 days) but missed integrated TAT (median 19 days). 4 (8.3%) diagnoses were changed. This indicates a need for improvement in integrated TAT, and suggestions for improvement mostly involved improving the efficiency of genetic testing.

P28

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A novel BMI1/Ephrin connection in human glioblastomas

Introduction: Epigenetic deregulation appears increasingly important in glioblastoma (GBM). BMI1 epigenetically silences downstream targets by inducing chromatin compaction and inhibiting transcription. In mouse models we have previously identified targets of Bmi1-mediated repression using genome-wide screening, and for EfnA5 this was functionally validated, with significant effects seen on proliferation, migration and invasion. We have begun to assess the translational value of these novel findings in human GBM.

Methods and results: We examined a cohort of 13 patient-derived GBM initiating cell cultures (hGBM-IC) by RNAseq and found a significant negative correlation between BMI1 and EFNA5, which was recapitulated when two further published RNAseq datasets were assessed. In our cohort, hGBM-IC were compared to matched iNSC and we observed that the Ephrin Receptor signalling pathway was significantly enriched in 70% of lines, was in the top 30 deregulated pathways in 30% and most significantly deregulated in the GBM with highest BMI1 expression. When published single-cell RNAseq datasets were analysed we found that a subgroup of human embryonic neural progenitors and a subset of GBM cells displayed a BMI1high; EFNA5low expression signature. Independent hGBM-IC from the Human Glioblastoma Cell Culture (HGCC) resource showed that upon BMI1 knockdown with shRNA, EFNA5 levels increased with a corresponding decrease in proliferation, whilst concomitant blockade of the EFNA5 signalling pathway rescued the phenotype.
Conclusions: We present evidence from human expression datasets and primary cells that the BMI1-EFNA5 pathway plays a prominent regulatory role in a proportion of hGBM-IC. Experiments confirming the importance of the connection in vivo are currently underway, with xenograft experiments and human surgical tissue analysis ongoing.

Multiple sclerosis and miscellaneous

P29

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Cerebral small vessel disease in multiple sclerosis

Introduction: Inflammation and BBB dysfunction feature in Multiple Sclerosis (MS) and may impact cerebral small vessel disease (SVD). However, the relationship between MS and cerebral SVD has not been explored. We aimed to compare the extent of cerebral SVD in MS and non-MS.
Material and methods: A human post-mortem cohort of MS and age- and sex- matched non-MS cases was assessed for systemic vascular disease (VD). Outside-plaque cerebral SVD was scored from formalin-fixed paraffin-embedded sections of the frontal and occipital white-matter, basal-ganglia, and pons stained with Hematoxylin & Eosin, using established criteria. Individual SVD pathologies (arteriolosclerosis, periarteriolar space dilatation (PSD) and hemosiderin deposition (PHD)) and a global SVD score were compared between MS and non-MS groups using multiple regression controlling for age, sex and VD.
Results: Forty-two (60.6 12.9 years, 57.1% females) MS cases and 39 (58.8 12.9 years, 56.5% females) non-MS cases were included. Global SVD increased with age (Exp(B) = 1.016, 95% CI 1.006,1.025, $P = 0.001$) and VD (Exp (B) = 1.65, 95% CI 1.099, 2.502, $P = 0.016$), MS having little impact on SVD (Exp(B) = 1.65, 95% CI 0.968, 2.83, $P = 0.06$). VD had a stronger effect on arteriolosclerosis in MS (b = 1.77, 95% CI 0.65–2.88, $P = 0.002$) compared to non-MS (b = 1.09, 95% CI −0.055–2.23, $P = 0.062$) and also on PSD in MS (b = 1.082, 95% CI −0.26–2.13, $P = 0.045$) but not in non-MS (b = 0.997, 95% CI −0.156–2.15, $P = 0.09$). PHD was more common in MS than non-MS ($P = 0.03$) while micro-infarcts were more common in non-MS cases ($P = 0.02$). Other SVD pathologies did not differ between the groups.
Conclusion: For the same amount of VD, MS cases have more SVD compared with non-MS cases. MS was related to PSD and PHD but not any of the other individual SVD pathologies.

P30

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Axonal protection by teriflunomide in an experimental lesion of Pattern III demyelination

Introduction: An energy deficit arising from inflammation-induced tissue hypoxia is believed to contribute to demyelination and axonal degeneration in multiple sclerosis (MS). Terifluonomide, a therapy for relapsing-remitting MS, inhibits the enzyme dihydroorotate dehydrogenase (DHODH), thereby limiting pyrimidine and DNA synthesis. The limitation can reduce the proliferation of lymphocytes, but also limit the mitochondrial DNA required for mitogenesis. Teriflunomide may

therefore limit mitochondrial formation in cells such as neurons that have high mitochondrial turnover. This limitation could exacerbate an energy crisis and associated pathology, but by inhibiting mitochondrial biogenesis before the onset of an inflammatory lesion, the drug could counterintuitively protect the tissue by invoking a preconditioning response that defends the cells from impending hypoxic energy crisis. We have shown the importance of tissue hypoxia in forming an experimental Pattern III demyelinating lesion, like those in MS1, and now use this lesion to explore the neuroprotective potential of teriflunomide.

Materials and Methods: Focal inflammatory demyelinating lesions were induced in male Sprague Dawley rats via the microinjection of lipopolysaccharide (LPS, 100 ng/μl) into the spinal dorsal columns. Oral administration of teriflunomide or vehicle (carboxymethylcellulose/tween) commenced 3 days before lesion induction and continued until perfusion fixation at 14 days. Lesion characteristics were examined by ex vivo MRI and histology.

Results: Treatment with teriflunomide did not reduce lesion size, or demyelination, but treated animals exhibited significantly more surviving axons (SMI-312 + ; $P < 0.01$), and more surviving damaged (SMI-32 +) axons, than vehicle-treated controls.

Conclusions: The data indicate that teriflunomide may protect demyelinated axons from degeneration. Axonal survival would be expected to promote functional recovery.

P31

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CLIPPERS: chronic lymphocytic inflammation with pontine perivascular enhancement responsive to steroids. A case study and review of neuropathological findings

Chronic Lymphocytic Inflammation with Pontine Perivascular Enhancement Responsive to Steroids (CLIPPERS) is a fairly recently described inflammatory disease predominantly affecting the brainstem and cerebellum with distinct histopathological, radiological and clinical features.

The histopathological findings include an angiocentric lymphocytic infiltrate composed predominantly of CD3-positive T-lymphocytes, a smaller number of CD68-positive histiocytes and CD20-expressing B-lymphocytes, with activated microglia. Importantly, there are no Langerhans giant cells nor non-necrotising granulomas and myelin is intact. Immunostaining for fungi, toxoplasma, CMV, EBV and JC virus is negative.

The aetiology of this rare disease remains poorly understood. However, with advances in immunopathology and radiology, CLIPPERS is recognised as a distinct entity that differs considerably in its clinical presentation, immunological characteristics, radiological findings and its responsiveness to steroids.

We describe a patient who presented with left facial numbness and dizziness and an MRI showing an enhancing lesion in the left cerebellar peduncle. Histopathological findings from 3 biopsies were supportive of CLIPPERS. The patient had a good outcome with long term immunosuppression and has been followed up for the past 8 years.

Neurodegeneration

P32

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Are visual hallucinations in Parkinson's disease a result of decreased perfusion of visual processing areas of the brain?

Background: Parkinson's disease (PD) is a common neurodegenerative disorder, in which patients frequently suffer from dementia. Up to 1/3 of patients with PD experience visual hallucinations (VH), which can be very distressing.

Neuroimaging studies suggest that perfusion is reduced in the occipital lobe in those with VH, but as Lewy bodies are sparse in this region they seem unlikely to explain the hallucinations. Recent work

suggested that decreased cholinergic input may lead to the decreased perfusion.

We hypothesised that individuals with Parkinson's disease and visual hallucinations would have biochemical evidence of reduced perfusion associated with reduced cholinergic activity in areas of the brain which process visual images.

Methods: We obtained tissue from BA18 and BA19, from 11 individuals with PD but not VH, 9 with PD and VH, 16 with PD dementia and VH, and 25 controls. The groups were matched for age, gender and post-mortem interval. We measured von Willebrand factor, vascular endothelial growth factor, myelin-associated glycoprotein:proteolipid protein-1 (MAG:PLP1, a measure of tissue oxygenation relative to metabolic demand), acetylcholinesterase, butyrylcholinesterase and α-synuclein by ELISA. The MAG:PLP ratio was the primary outcome measure.

Results: There was no evidence of chronic hypoperfusion in PD ($F = 0.7184$, $P = 0.54$). There was no between-group difference in butyrylcholinesterase in dorsal BA18 or BA19. Acetylcholinesterase concentration was reduced in all three PD groups ($F26.83$, $P < 0.001$) which was not related to disease duration.

Conclusions: We have not found evidence that chronic hypoperfusion of visual processing areas in the occipital cortex causes VH in PD. Further investigation of the cholinergic data is in progress.

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Do anti-cholinergic drugs increase Alzheimer-type pathology in Parkinson's patients? A retrospective post-mortem investigation

Introduction: Medical management of Parkinson's disease (PD) is complex, with some patients on multiple medications for motor and non-motor symptoms control. Many of these medications have anti-cholinergic properties. Accumulated anti-cholinergic drug (ACD) use has been associated with an increased risk of dementia (1). However, only one post-mortem study has investigated the effects of ACD on Alzheimer-type pathology (2), with equivocal results. Therefore, we have undertaken a retrospective study to determine if we see a correlation between cumulative ACD use and the amount of Alzheimer-type pathology seen in post-mortem PD brains.

Material and methods: Clinical notes from 54 PD cases from the Parkinson's UK Brain Bank were retrospectively analysed. Using literature-based anti-cholinergic scores with the duration of medication use, cases were stratified into No, Low, and High anti-cholinergic burden. Presence of Alzheimer-type pathology was recorded from tau and amyloid-beta (Aβ) immunostained brain sections. Semi-quantitative assessment for tau was carried out on sections used for Braak tau staging.

Results: A higher anti-cholinergic burden is associated with greater tau burden in entorhinal cortex in PD cases ($P = 0.047$; OR = 2.21; 95% CI 1.01–4.85). Interestingly, a high anti-cholinergic burden decreases the odds of Aβ in the anterior hippocampus ($P = 0.047$; OR = 0.127; 95% CI = 0.017–0.969), entorhinal cortex ($P = 0.037$; OR = 0.124; 95% CI = 0.017–0.885) and frontal cortex ($P = 0.031$; OR = 0.113; 95% CI = 0.016–0.819) of PD cases.

Conclusion: ACD have varying effects on Alzheimer-type pathology, depending on the brain region. High anti-cholinergic burden is associated with increased tau but less Aβ in the entorhinal cortex.

References:

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2. Perry et al. Annals of neurology 54.2 (2003): 235-238

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Can early tau depositions in mixed Alzheimer's disease and Lewy body disease give insights into disease progression?

Cases that neuropathologically fulfil the criteria for both Alzheimer's disease (AD) and Lewy body disease (LBD) are classified as mixed dementia (mixed AD/LBD). Interestingly, some of these cases present clinically with AD, and others with LBD.

Previous work indicates cases with an AD clinical phenotype exhibit a higher hyperphosphorylated tau

burden [1]. This may suggest that tau pathology has been developing for a longer period of time and is more established in these cases. Conversely, in cases with a LBD clinical phenotype hyperphosphorylated tau may have been deposited at a later time point in LBD progression and still in the early stages of development.

The aim of this project was to determine if mixed AD/LBD cases that presented clinically with LBD have a higher burden of tau in the early stage of development. We quantitatively assessed post-mortem tissue sections from several brain regions from cases that have mixed AD/LBD, with tau marker MC1 to identify early tau conformations. We also assessed markers of more established tau pathology including AT8 and CP13.

Mixed AD/LBD cases with a LBD clinical phenotype had a greater MC1 burden in the hippocampus and temporal lobe ($P < 0.05$) compared to those with an AD clinical phenotype, suggesting LBD may be the initial cause of dementia, and concomitant AD related pathology developed later in the disease course. These results highlight the importance of biomarkers for comorbid pathologies and if identified, secondary pathologies should be considered in future treatment strategies.

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Serotonergic ^{123}I-FP-CIT binding is associated with depression and not neuropathology

Objectives: The objective of the study was to investigate the influence of dorsal and median raphe pathology on ^{123}I-N-ω-fluoropropyl-2β-carbomethoxy-3β-(4-iodophenyl)nortropane (^{123}I-FP-CIT) serotonin transporter (SERT) binding in a cohort of post-mortem confirmed Alzheimer's disease (AD) ($n = 6$), dementia with Lewy bodies (DLB) ($n = 6$), Parkinson's disease with dementia (PDD) ($n = 11$), mixed AD/DLBs ($n = 5$) and healthy-aged controls ($n = 5$).
Methods: Subjects underwent ante-mortem serotonergic ^{123}I-FP-CIT single photon emission computed tomography (SPECT), neuropsychiatric testing including, Mini-Mental State Examination and Geriatric Depression Scale (GDS) and post-mortem assessments (mean interval 6.6 years). SERT binding was estimated using region of interest procedures, with quantitative neuropathological analysis of alpha-synuclein, tau and amyloid-beta.
Results: SERT binding ratios were significantly lower in PDD than control patients ($P < 0.01$). No statistical differences in pathology, alpha-synuclein, tau or amyloid-beta, were found between PDD and DLB patents. GDS assessed at time of 123I-FP-CIT correlated with the SERT binding ratio ($P < 0.001$), with scores from PDD patients significantly higher than controls ($P < 0.01$).
Conclusions: The results suggest that the differences observed in ^{123}I-FP-CIT SERT binding in PDD compared to controls ante-mortem, are related to the changes that underlie geriatric depression and not to the underlying pathology. Preliminary data from immunofluorescence suggests that serotonergic neurons are more vulnerable to Lewy body pathology than other neuronal subtypes in the raphe, suggesting a possible reason for the lowered SERT binding ratios observed.

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White matter hyperintensities and pathological correlations in Alzheimer's disease

Introduction: Magnetic resonance imaging (MRI) is a widely used tool to investigate Alzheimer's disease (AD). However, MRI markers are often non-specific and

observed signal changes commonly have heterogeneous pathological bases. One such marker is white matter hyperintensities (WMH), with studies suggesting numerous histopathological correlates, including ependymal loss, cerebral ischaemia and demyelination. This methodological study examines the effectiveness of a pipeline that includes both post-mortem imaging and histological investigations to elucidate the pathological basis of specific WMH.

Material and methods: Fixed left hemispheres of both sporadic (n = 3) and familial AD patients (n = 1) were imaged in a Siemens 3 T scanner to obtain T2-weighted, susceptibility-weighted and diffusion-weighted imaging. Hemispheres were then sampled using a protocol designed to aid future registration of brain slices back to the post-mortem MRI images. The protocol was also designed to enable preservation of both WMH and normal appearing white matter for immunohistochemical staining to be carried out for markers of interest.

Results: High quality post-mortem images were obtained for each imaging modality, as well as the successful sampling and preservation of brain regions of interest for future immunohistochemical investigations.

Conclusions: Our pipeline was successful in enabling the scanning and sampling of brain regions of interest to aid the discovery of the pathological underpinnings of signal changes seen on MRI.

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DNA hydroxymethylation in amyotrophic lateral sclerosis (ALS)

Introduction: ALS is a progressive neurodegenerative disease characterised by motor neuron (MN) degeneration, resulting in motor impairment and muscle wasting. Around 10% of ALS cases are familial, including cases caused by C9orf72 mutations (C9ALS), and the other 90% are sporadic (sALS). DNA can undergo methylation, wherein a cytosine is methylated to form 5-methylcytosine (5mC), followed by hydroxymethylation, forming 5-hydroxymethylcytosine (5hmC), both resulting in gene silencing. These processes have been implicated in neurodegeneration and data from our lab has implicated methylation in ALS. This study investigated pathological hydroxymethylation in ALS spinal cord.

Materials and Methods: Immunohistochemistry for 5hmC was performed in cervical spinal cord in three groups: control, sALS and C9ALS (n = 10 in each). Analysis was conducted in three regions: anterior horn (AH), lateral corticospinal tract (LCT) and dorsal column (DC).

Results: Inter-rater reliability testing gave a 98% agreement. There was no significant difference between controls and ALS cases in the number of glia positive for 5hmC. However, there was a significant difference between control and ALS cases in the number of MNs positive for 5hmC: Control cases had an average of 82% (SD = 5.73) 5hmC-positive MNs in the AH. There were greater numbers of 5hmC-positive nuclei in sALS cases (mean = 90%, SD = 3.77, P = 0.0011) and C9ALS cases (mean = 91%, SD = 4.68, P = 0.00072).

Conclusions: MNs, but not glia, show greater levels of hydroxymethylation in ALS.

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Increased calcyclin immunoexpression in ALS is seen within reactive astrocytes in corticospinal tracts and is not specific for ALS subtype

Introduction: Mutations in the Annexin A11 (ANXA11) gene account for a small proportion of familial Amyotrophic Lateral Sclerosis (FALS) cases. However, a common functional outcome of ANXA11 disruption is abolished binding to the calcium binding protein - calcyclin. An ANXA11 mutant case displayed distinct over-expression of calcyclin protein in astrocytes of the

corticospinal tracts of spinal cord but not in the anterior horn neurones. The purpose of the study was to investigate whether this immunohistochemical pattern was also seen in cases of sporadic ALS (SALS) and FALS with different mutations.

Materials and Methods: Paraffin sections from spinal cord and medulla from eleven cases of FALS of different types, four cases of SALS, three cases of disease controls (multiple sclerosis, subacute combined degeneration of the cord and multiple system atrophy) and six non-disease control cases were stained with antibodies against calcyclin.

Results: The control cases showed minimal astrocytic staining for calcyclin. The SALS and FALS cases revealed mainly strong astrocytic immunopositivity for calcyclin in the corticospinal tracts of the cord and variable staining in the pyramids of the medulla. The degree of staining appeared to reflect the overall severity of the corticospinal tract degeneration in the individual cases. The disease control cases showed mainly astrocytic/glial staining in the lesions characteristic of each disease.

Conclusions: The study results suggest that calcyclin immunoexpression is seen in reactive astrocytes in ALS, with no specific difference in staining pattern seen between different FALS or SALS cases, and that calcyclin over-expression is also seen in other neurological conditions in which there is significant astrocytosis/gliosis present.

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Clinical and pathological features of multiple system atrophy and multiple system atrophy lookalikes

Clinical diagnosis of multiple system atrophy (MSA) is challenging and patients may be misdiagnosed as having Parkinson's disease (PD) or progressive supranuclear palsy (PSP).

The clinical records of 203 patients with a clinical diagnosis of MSA were reviewed to identify diagnostic pitfalls. We also examined twelve red flag features that support the diagnosis of MSA and assessed disease progression using seven disability milestones. 160 cases (78.8%) had pathologically confirmed MSA. The remaining 21.2% (43/203) had other pathological diagnoses including PD (12.8%; $N = 26$), PSP (6.4%; $N = 13$), cerebrovascular diseases (1%; $N = 2$), amyotrophic lateral sclerosis (0.5%; $N = 1$) and cerebellar degeneration (0.5%; $N = 1$). More MSA patients developed dysphagia, stridor and falls than PD patients, while pill-rolling tremor and hallucination were more frequent in PD. Ataxia and stridor were more common in MSA than in PSP. Multiple logistic regression analysis revealed increased likelihood of MSA if a patient developed orthostatic hypotension and/or urinary incontinence with urinary catheterisation (MSA vs PD: odds ratio (OR): 2.0, 95% confidence interval (CI): 1.1–3.7, $P = 0.021$; MSA vs PSP: OR: 11.2, 95% CI: 3.2–39.2, $P < 0.01$). Patients with MSA-parkinsonian had more red flags than patients with PD (OR: 8.7, 95% CI: 3.2–24.0, $P < 0.01$) and PSP (OR: 4.6, 95% CI: 1.6–12.9, $P < 0.01$). The number of red flags in MSA-cerebellar was higher than in PD (OR: 6.9, 95% CI: 2.5–19.1, $P < 0.01$) and PSP (OR: 3.0, 95% CI: 1.1–8.5, $P = 0.035$). Compared with MSA patients, PD patients required more time to reach the following milestones: frequent falls, unintelligible speech and cognitive impairment. The present study has highlighted features that will improve the diagnostic accuracy of MSA.

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Investigation of tau seeding activity in tauopathies

In recent years, it has been proposed that transcellular propagation (seeding) of disease-associated tau in a prion-like manner, may underlie the spread of pathology in tauopathies. Studies investigating tau seeding in animal and mammalian cell-based models have provided some insight into tau seeding activity and spreading in Alzheimer's disease (AD) pathology. However, there remains much to be uncovered regarding tau seeding mechanisms in AD and in other tauopathies such as corticobasal degeneration (CBD) and progressive supranuclear palsy (PSP).

In this presentation we demonstrate that we have utilised FRET (fluorescence resonance energy transfer)

combined with flow cytometry for the quantification of the seeding activity of brain homogenates from tauopathies including AD, CBD and PSP. For this we used HEK293 biosensor cells stably expressing the repeat domain of tau tagged with cyan fluorescence protein/yellow fluorescence protein (CFP/YFP-tagged RD-tau) allowing for the detection of tau seeding activity. Flow cytometry was used to identify and record the aggregation induced FRET signals caused by tau proteopathic seeds from the different tauopathies within single cells. Our initial data demonstrates that this methodology is a powerful tool for the study of the seeding potential of different tauopathies. We intend to apply this methodology for the investigation of large cohorts of cases.

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The expression and presence of RNA binding proteins in FTLD-TDP

Frontotemporal lobar degeneration (FTLD) is pathologically classified according to the protein present in the inclusions; these include tau, 43 kDa transactive responsive DNA-binding protein (TDP-43) and fused in sarcoma (FUS). The mechanisms causing FTLD are still unknown, but insights have been gained from those identified with mutations in specific genes. Pathologically FTLD-TDP is classified into four subtypes A, B, C and D according to the characteristics and anatomical location of their hyper-phosphorylated TDP-43 positive inclusions. TDP-43 belongs to the heterogeneous nuclear ribonucleoprotein (hnRNP) family and is hyper-phosphorylated forming ubiquitin positive inclusions underlying FTLD-TDP pathology. HnRNP E2 and A3 have already been shown to distinguish different pathological FTLD-TDP subtypes and therefore we have investigated whether the expression of other hnRNPs were altered in these subtypes and whether these proteins could be identified in FTLD-TDP inclusions. We used nanostring technology and immunohistochemical techniques to investigate 10 hnRNPs (hnRNP C-I, L, M and U) in the frontal cortex and hippocampus from post-mortem human brain tissue from three different FTLD-TDP subtypes (A, B and C). Expression analysis showed an increase in several hnRNPs in FTLD-TDP. However, none of the 10 hnRNPs tested within the frontal cortex and hippocampus were localised within the TDP-43 positive inclusions across all FTLD-TDP subtypes shown using double immunofluorescent staining. This highlights the pathological mechanisms involved in FTLD-TDP may involve different proteins even though these were not identified in the pathological inclusions. Implicating that RNA binding proteins may play a wider role in the dysfunction of RNA movement between intracellular compartments in these diseases.

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A single cell omics approach to neurodegeneration

Single cell RNA sequencing allows analysis of a cell's specific function and state and is a powerful tool in understanding what is occurring within a cell in relation to the larger complex environment. Allying this technology to laser capture microdissection (LCM) allows transcription of a cell to be analysed in relation to its position. Single cell technologies are advancing and are now being applied to neurodegeneration.

LCM was used to extract motor neurones (MNs) from FFPE mouse spinal cord, followed by RNA extraction and NGS library preparation. 1, 5, 10 and 35 cells were collected to optimise and determine the limitations of the single cell omics approach.

Roche High Pure FFPET RNA extraction kit delivered the most consistent results when compared to other kits. Nanodrop and Agilent PicoChip were used to determine the quantity and quality of RNA extracted. Mean 260/280 and 260/230 ratios of 1.38, 0.68 were determined with 6–36 pg/l of RNA and RIN numbers between 0 and 3.7. A sequencing library was produced with Qiagen FX single cell library kit. QuBit fluorometer and a high sensitivity DNA chip were used to check quality and quantity. The final library had 8.7 nM, 4.4. nM, 3.2 nM and 0.16 nM for 1, 5, 10 and 35 cells respectively. Samples also underwent PCR to

determine if only MNs were present, as well as qPCR to check adapter ligation.

This method will be applied to selective vulnerability in motor neurons for ALS, utilising FFPE tissue from TDP-43 mutant mice. This will allow for direct comparison of gene expression levels between individual cells.