# 120th Meeting of the British Neuropathological Society
# Mary Ward House Conference and Exhibition Centre, 5-7 Tavistock Place, London WC1H 9SN

## WEDNESDAY 6th March, 2019

## Developmental Neuropathology Symposium

Chair: Professor Tom Jacques,
*UCL Great Ormond Street Institute of Child Health, UK*

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|---|---|
| 12:30 | Registration |
| 13:25 | **Welcome and Introduction** - Tom Jacques |
| 13:30 | **Interneurons in Cerebral Cortical Developmental Disorders**<br>Jeffrey Golden, Brigham and Women's Hospital and Harvard Medical School Boston, USA |
| 14:15 | **Leukodystrophies**<br>Marianna Bugiani, VU Medical Center, Amsterdam, The Netherlands |
| 15:00 | Tea Break |
| 15:30 | **Neurodegeneration with Brain Iron Accumulation (NBIA): Genetics, Neuropathology and Novel Treatments**<br>Manju Kurian, UCL Great Ormond Street Institute of Child Health, UK |
| 16:15 | **Preterm brain injury: mechanistic insights from magnetic resonance imaging**<br>James Boardman, University of Edinburgh, UK |
| 17:00 | Short Break |
| 17:15 | **Alfred Meyer Memorial Lecture - Neural Tube Defects**<br>Andrew Copp, UCL Great Ormond Street Institute of Child Health, UK |
| 18:15 | Reception |

## THURSDAY 7th March, 2019

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| 08:00 | Registration | |
| 08:50 | **Opening of meeting – Professor Colin Smith, BNS President** | |
| 09:00 – 10:30 | **FIRST SCIENTIFIC SESSION – cerebrovascular disease** | |
| | **Chairs: Stephen Wharton, Roxana Carare** | |
| 9:00 | *A. Willetts*, J. Booker, M. Gatherer, N. Albargothy, A. Verma, R. Weller, R. Carare, C. Hawkes<br>**Pathways for flow of solutes along walls of cerebral arteries are maintained even in the presence of Cerebral Amyloid Angiopathy** | O01 |
| 9:15 | *E. Norton*, L. Bridges, L. Kenyon, M. Esiri, D. Bennett, A. Hainsworth<br>**Mural cell depletion and senescence in cerebral small vessel disease: a neuropathology study in older people (aged 80+)** | O02 |
| 9. 30 | *M. Goldfinger*, B. Tilley, M. Sastre, S. Gentleman<br>**A tale of two tauopathies: a comparison of vasculature changes in ARTAG and chronic traumatic encephalopathy** | O03 |
| 9:45 | *M. Frost*, A. Sealy, M. Gatherer, C. Smith, J. Attems, R. Weller, R. Carare<br>**Loss of adrenergic receptors on cerebral blood vessels may contribute to the accumulation of Amyloid-β in Cerebral Amyloid Angiopathy** | O04 |
| 10.00 | H. Tayler, R. MacLachlan, I. Schulz, O. Skrobot, S. Miners, S. Love<br>**Post-mortem biochemical markers of vascular dysfunction: associations with midlife hypertension, VCING, endothelin- 1 and dementia** | O05 |
| 10:15 | *D. Hansen*, T. Lashley, J. Holton, T. Warner<br>**Cerebral amyloid angiopathy in Lewy body dementias** | O06 |
| 10:30 –11:00 | **Coffee break and POSTER DISCUSSION** | |
| 11:00– 12:15 | **SECOND SCIENTIFIC SESSION – Alzheimer's disease** | |
| | **Chairs: Tammaryn Lashley, Johannes Attems** | |
| 11:00 | *C. Toomey*, S. Foti, W. Heywood, K. Mills, T. Lashley<br>**Proteomic differences between Alzheimer's disease and Frontotemporal dementia human post-mortem brains** | O07 |
| 11.15 | *H. Mistry*, J. Simpson, A. Chambers, C. Garwood, P. Ince, P. Heath, S. Wharton<br>**Cholesterol dysregulation in the neurone-astrocyte unit as an early contributor to neuronal dysfunction in Alzheimer's Disease** | O08 |
| 11:30 | *Z. Jaunmuktane*, A. Quaegebeur, R. Taipa, S. Mead, S. Brandner<br>**Human transmission of amyloid-β cerebral amyloid angiopathy** | O09 |
| 11:45 | *K. McAleese*, S. Graham, M. Dey, L. Walker, D. Erskine, M. Johnson, A. Thomas, McKeith, C. DeCarli, J. Attems<br>**Fibrinogen leakage in the white matter of Alzheimer's disease and normal aged brains: Implications for fibrinogen as a biomarker** | O10 |
| 12:00 | *R. Waller*, R. Narramore, J. Simpson, F. Matthews, C. Brayne, P. Ince, R. Kalaria, S. Wharton | O11 |

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| | **Heterogeneity of Cellular Inflammatory Responses in Ageing White Matter and Relationship to Alzheimer's and Small Vessel Disease Pathologies** | |
| 12:15 – 13:00 | **Lunch** | |
| 13.00 –14.00 | **John Cavanagh Prize Lecture, introduced by the President, Colin Smith**<br>**Epigenetic mechanisms in dementia**<br>Katie Lunnon, University of Exeter | |
| 14.00 – 15.45 | **THIRD SCIENTIFIC SESSION – Neurodegeneration** | |
| | Chair: Seth Love, Stephen Gentleman | |
| 14:00 | *M Foiani*, T. Jackson-Morgan, C. Cicognola, N. Ermann, K. Ye, J. Kornhuber, N. Fox, P. Lewczuk, H Zetterberg, K. Blennow, K. Hoglund[3], J. Rohrer[5], T. Lashley<br>**Profiling of tauopathies in frontotemporal dementia** | O12 |
| 14:15 | *S. Kaalund*, K. Allinson, M. Spillantini, J. Rowe<br>**Quantification of neurons in the inferior frontal gyrus in frontotemporal lobar degeneration** | O13 |
| 14:30 | *B. Tilley*, M. Goldfinger, R. Pearce, S. Gentleman<br>**The role of tau and basal ganglia cholinergic pathology in the pathogenesis of Parkinson's disease motor subtypes** | O14 |
| 14:45 | B. Sonustun, C. Strand, S. Foti, T. Warner, H. Lashuel, J. Holton, *R. Bandopadhyay*<br>**Investigation of alpha-synuclein post-translational modifications in idiopathic Parkinson's disease and multiple system atrophy** | O15 |
| 15.00 | *C. Bettencourt*, S. Foti, T. Lashley, R. Balazs, E. Vire, J. Holton<br>**DNA methylation landscapes in patients with Multiple System Atrophy** | O16 |
| 15:15 | *C. Appleby-mallinder*, P. Heath, R. Highley<br>**DNA methylation in Amyotrophic Lateral Sclerosis (ALS)** | O17 |
| 15:30 | *Richard Cain*, Laura Palmer, Seth Love<br>**UK Brain Banks Network Database: a resource for researchers and brain banks** | O18 |
| 15:45 – 16:15 | **Coffee Break and POSTER DISCUSSION** | |
| 16:15 – 17:00 | **BUSINESS MEETING** | |
| 17:00 – 18:00 | **PROFESSIONAL AFFAIRS MEETING** | |
| 19:30 | **SOCIETY DINNER AT THE CRYPT** | |

## FRIDAY 8th MARCH 2019

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| 08:00 | registration | |
| 9:00 – 10:30 | **FOURTH SCIENTIFIC SESSION – Brain tumours** | |
| | **Chairs: Sebastian Brandner, Kathreena Kurian** | |
| 9:00 | **S. Nagaraju, *U. Pohl*** | |
| | **Characterisation of gliomas with rare IDH mutation at QEHB during 2016-2018** | O19 |
| 9:15 | K. Loveson, P. Singh, K. Allinson, *H. Fillmore* | |
| | **Paediatric brainstem glioma and its intersection with brain development: role of tumour microenvironment** | O20 |
| 9:30 | **I. Smolicz, A. Fairchild, J. Pickles, T. Stone, J. Chalker, J. Gonzalez Zapata, L. Wilkhu, S. Yasin, D. Hargrave, N. Sebire, T. Jacques** | |
| | **The biology of paediatric brain tumours at post-mortem: a national cohort** | O21 |
| 9:45 | *L. Laraba*, S. Ferluga, E. Ercolano, C. Adams, A. Shivane, P. Edwards, M. Futschik, V. Lenis, J. Grimm de Guibert, S. Moller, O. Hanemann, D. Parkinson | |
| | **The Cancer Stem Cell Marker ALDH1A1 is Upregulated and Drives Proliferation in Merlin Null Meningioma and Schwannoma** | O22 |
| 10:00 | *O. Curran*, L. Gilroy, A.Torgersen, C. Smith, W. Al-Qsous | |
| | **Intracerebral lymphoid malignancies: a retrospective study of morphological, immunohistochemical and molecular characteristics with survival correlation** | O23 |
| 10:15 – 10:45 | Coffee break | |
| 10:45 – 12:00 | **FIFTH SCIENTIFIC SESSION - Neuroinflammation** | |
| | **Chairs: Colin Smith, Robin Highley** | |
| 10:45 | *J. Spencer*, J. Watson, G. Niblett, Y. Mahjoub, R. Yates, G. Hadley, C. Brendler-Spaeth, B. Kessler, R. Fischer, G. DeLuca | |
| | **Extracellular matrix proteins interface with HLA genotype to modulate topographical variation of multiple sclerosis pathology** | O24 |
| 11:00 | *B. Ashford*, C. Appleby-Mallinder, P. Heath, J. Simpson, R. Highley | |
| | **The Role of Immunity in Human Motor Neuron Disease** | O25 |
| 11:15 | *D. Munoz*, M. Abdollahi | |
| | **The Structural Basis of Susac's Syndrome** | O26 |
| 11:30 | *R. Raha-chowdhury*, J. Henderson, A. Raha, E. Jones, R. Fincham, K. Allinson, A. Holland, S. Zaman | |
| | **Choroid plexus acts a gatekeeper for TREM2, abnormal accumulation of ApoE and fibrillary tau in Alzheimer's disease and Down's syndrome** | O27 |
| 11.45 – 12:15 | Lunch | |
| 12:15 – 13:15 | POSTER DISCUSSION | |
| 13:15 – 14:00 | Clinical update session: | |
| | **Tumours of the sellar region - update on classification and new entities** | |
| | Federico Roncaroli | |

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| **14:00 – 14:30** | DIAGNOSTIC SLIDE SESSION (INCORPORATING EQA) |
| **14:30 – 15:00** | break |
| **15:00 – 16:15** | **SIXTH SCIENTIFIC SESSION – developmental neuropathology, epilepsy and skeletal muscle** |

## Chairs: Janice Holton, Maria Thom

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|---|---|---|
| **15:00** | *L. Chareyron,* D. Gadian, F. Vargha-Khadem<br>**Amygdala development in patients with hippocampal atrophy: associations with socioemotional status** | O28 |
| **15.15** | *K. Long,* B. Newland, M. Florio, N. Kalebic, B. Langen, A. Kolterer, P. Wimberger, W. Huttner<br>**How does the human neocortex fold? A novel role of the Extracellular Matrix** | O29 |
| **15.30** | A. Sarkozy, S. Torelli, P. Ala, D. Ardicli, L. Feng, R. Mein, S. Aguti, H. Zhou, C. Sewry, *R. Phadke,* F. Muntoni<br>**Quantitative flow cytometry in the diagnosis of Collagen VI-related disorders: 3-year experience from a tertiary referral centre** | O30 |
| **15.45** | *D. Chambers,* A. Kumar, L. Feng, I. Hargreaves, A. Lam, S. Heales, A. Manzur, F. Muntoni, C. Sewry, J. Poulton, R. Phadke<br>**A novel multiplex chromogenic immunoassay for evaluating mitochondrial respiratory chain complex I and complex IV defects in diagnostic muscle biopsies** | O31 |
| **16.00** | *J. Liu*, M. Ellis, M. Thom, S. Sisodiya<br>**Cellular expression of SCN1A mRNAs in patients with temporal lobe epilepsy** | O32 |
| **16.15** | Announcement of the poster prize winners and closure of the meeting | |

## Posters

**Microglia in cortical and subcortical autonomic brain regions in SUDEP** P01
*M. Thom*, A. Somani, S. Sisodiya
**Volumetric and neuropathological study of the medulla in SUDEP and correlates with 9.4T MRI** P02
M. Thom, *S. Patodia*, S. Sisodiya
**Medullary catecholaminergic neurones in Sudden Unexpected Death in Epilepsy** P03
M. Thom, *S. Patodia*, I. Tan, S. Sisodiya
**Adenosine kinase and Adenosine receptors A1 and A2A in temporal lobe epilepsy and association with risk factors for SUDEP** P04
M. Thom, *B. Paradiso*, S. Patodia, M. Garcia, B. Diehl, M. Ellis, O. Devinsk
**Identification of miRNA and mRNA regulatory networks in the ageing blood-brain barrier: Comparative gene expression studies in human and mouse** P05
**V. Leach**, E. Goodall, J. Cooper-Knock, C. Wang, J. Simpson, D. Baker, D. Drew, M. Saffrey, I. Romero, P. Heath, S. Wharton
**The effect of systemic atherosclerosis in the neurovascular unit** P06
**M. Alejandra Rebollar**, S. Wharton, S. Francis, J. Simpson
**Carotid Artery Disease, Strokes and Experimental Effects of Enriched Environment on Stroke Injury** P07
**Y. Hase**, T. Polvikoski, M. Hase, W. Stevenson, M. Ihara, L. Allan, K. Horsburgh, R. Kalaria
**Adrenergic receptors in the walls of cerebral vessels as possible targets for improving Intramural Peri-Arterial drainage in CAA** P08
A. Keable, D. Baseley, D. Zbarcea, M. Gatherer, D. Johnston, C. Smith, J. Attems, R. Weller, R. Carare
**Changes in the Intramural Peri-Arterial Drainage (IPAD) pathways after traumatic brain injury** P09
**V. Unadkat**, M. Gatherer, A. Pringle, A. Ahmed, R. Weller, R. Carare
**Iba-1-/CD68+ microglia are a prominent feature of age-associated deep subcortical white matter lesions** P10
S. Waller, L. Baxter, D. Fillingham, S. Coelho, J. Pozo, M. Mozumder, A. Frangi, P. Ince, J. Simpson, **R. Highley**
**Microglial Dynamics in the Developing and Early Postnatal Human Brain** P11
**D. Menassa**, L. Barry-Carroll, J. Nicoll, M. Chapman, T. Bloom, S. Lisgo, I. Adorjan, Z. Krsnik, I. Kostovic, T. Jacques, O. Ansorge, D. Gomez-Nicola
**A versatile, modular digital script for automated high-throughput multiparametric myofibre analysis in brightfield and epifluorescent paradigms** P12
M. Ellis, D. Scaglioni, F. Catapano, V. Sardone, D. Chambers, L. Feng, E. Curtis-Wetton, S. Saeed, A. Sigurta, N. Hill, M. Singer, C. Sewry, S. Torelli, J. Morgan, F. Muntoni, **R. Phadke**
**Recessive loss-of-function mutations in ITGA7 cause cardiac arrhythmia with or without structural cardiomyopathy and respiratory muscle weakness** P13
E. Bugiardini, **R. Phadke**, R. Maas, A. Pittman, B. Kusters, J. Morrow, M. Parton, A. Nunes, M. Akhtar, P. Syrris, L. Lopes, T. Fotelonga, H. Houlden, P. Elliott, M. Hanna, J. Raaphorst, D. Burkin, E. Matthews
**Congenital fatal cap-rod myopathy due to a de novo autosomal dominant pathogenic ACTA1 variant** P14
**R. Phadke**, B. Herron, D. Hurrell, S. Craig, B. Kelly, R. Mein, A. Sarkozy, C. Sewry, F. Muntoni, V. McConnell
**Extended phenotypic spectrum of VCP inclusion body myopathy: report of two cases with atypical early and late childhood-onset disease** P15
**R. Phadke**, A. Dean, M. Evans, A. Parker, D. Maxwell, C. Sewry, A. Sarkozy, F. Muntoni
**Diagnostic challenges in paediatric anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase necrotising myopathy (anti-HMGCR-NM)** P16
**R. Phadke**, M. Pal-Magdics, C. Pilkington, S. Maltby, A. Ismail, A. Chakrabarty, P. Munot, M. Wood, A. Manzur, F. Muntoni, A. Sarkozy
**A novel case of MSTO1-related congenital muscular dystrophy with cerebellar involvement** P17
D. Ardicli, I. Zaharieva, C. Deshpande, I. Bodi, A. Siddiqui, J. Marie U-King-Im, **R. Phadke**, A. Sarkozy, H. Jungbluth, F. Muntoni
**Prevalence of cytoplasmic bodies in a large series of diagnostic paediatric muscle biopsies** P18
**M. Aizpurua Gomez**, L. Feng, F. Leiva-Cepas, A. Sarkozy, A. Manzur, F. Muntoni, C. Sewry, R. Phadke
**SLONM: Sporadic Late-Onset Nemaline Myopathy. A case study and review of neuropathological findings** P19
*C. Turnquist*, M. Hofer, D. Hilton-Jones
P20

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**Investigating senescence activation in response to oxidative DNA damage in neurones in vitro**
*I. Vazquez-Villasenor*, C. Garwood, P. Heath, J. Simpson, S. Wharton

**Advanced molecular characterization using Digital Spatial Profiling of immuno-oncology target expression in methylated versus unmethylated IDH-wildtype glioblastoma** P21
H. Barber, B. Lander, **A. Daniels**, A. Tofias, J. Gong, X. Ren, Y. Ren, P. White, K. Kurian

**Modelling high-risk paediatric brain tumour infiltration** P22
**A. Fairchild**, A. Rolland, Y. Li, T. Stone, J. Pickles, T. Jacques

**DNA methylation profiling in paediatric CNS tumours** P23
**J. Pickles**, *T.* Stone, L. Brownlee, A. Merve, S. Yasin, L. Wilku, M. Kristiansen, D. Hargrave, J Chalker, T. Jacques

**Characterisation of histone mutant gliomas in adults at QEHB 2015-2018** P24
**U. Pohl**, S. Nagaraju

**EGFR as a potential prognostic biomarker in adult IDH-wildtype Glioblastomas** P25
*C. Cabral*, R. Laxton, L. Doey, I. Bodi, A. King, L. Brazil, R. Bhangoo, K. Ashkan, S. Al-Sarraj

**A rare case of fibroblastic reticular cell tumour in the spine** P26
**Z. Reisz**, J. Salisbury, D. Bell, I. Bodi, S. Al-Sarraj

**Audit of turnaround time (TAT) in glioma diagnosis** P27
**Oliver Sargent**

**A novel BMI1/Ephrin connection in human glioblastomas** P28
*T. Millner*, B. Ricci, X. Zhang, N. Pomella, G. Rosser, S. Marino

**Cerebral small vessel disease in multiple sclerosis** P29
*R. Geraldes*, M. Esiri, J. Palace, G. DeLuca

**Axonal protection by teriflunomide in an experimental lesion of Pattern III demyelination** P30
R. Desai, A. Davies, M. Tachrount, X. Golay, **K. Smith**

**Are visual hallucinations in Parkinson's disease a result of decreased perfusion of visual processing areas of the brain?** P31
*L. Sinclair*, J. Brenton, A. King Lun Liu, S. Gentleman, S. Love

**CLIPPERS: Chronic Lymphocytic Inflammation with Pontine Perivascular Enhancement Responsive to Steroids. A case study and review of neuropathological findings** P32
*C. Turnquist*, M. Hofer, J. Halliday, R. Kerr

**Do anti-cholinergic drugs increase Alzheimer-type pathology in Parkinson's patients? A retrospective post-mortem investigation** P33
*A. King Lun Liu*, Y. Mun Lim, R. Pearce, S. Gentleman

**Can early tau depositions in mixed Alzheimer's disease and Lewy body disease give insights into disease progression?** P34
*L. Walker*, E. Thomson, M. Thamed, K. McAleese, M. Johnson, J. Attems

**Sertonergic $^{123}$I-FP-CIT binding is associated with depression and not neuropathology** P35
**A. Oliver-Evans**, S. Colloby, A. Thomas, D. Erskine, J. Attems

**White Matter Hyperintensities and Pathological Correlations in Alzheimer's Disease** P36
**P. Walsh**, D. Thomas, C. Sudre, J. Iglesias, S. Crampsie, Z. Jaunmuktane, J. Holton, N. Ryan, J. Barnes, T.Lashley

**DNA hydroxymethylation in Amyotrophic Lateral Sclerosis (ALS)** P37
**E. Schaber**, C. Appleby-Mallinder, R. Highley, P. Heath

**Increased calcyclin immunoexpression in ALS is seen within reactive astrocytes in corticospinal tracts and is not specific for ALS subtype** P38
**A. King**, C. Troakes, C. Shaw, V. Marchica, S. Al-Sarraj, B. Smith

**Clinical and pathological features of multiple system atrophy and multiple system atrophy lookalikes** P39
**Y. Miki**, H. Ling, S. Foti, J. Holton

**Investigation of tau seeding activity in tauopathies** P40
**T. Bradshaw**, H. Ling, J. Holton, S. Wray, R. de Silva, T. Revesz

**The expression and presence of RNA binding proteins in FTLD-TDP** P41
**S. Foti**, L. Gittings, C. Toomey, Y. Asi, T. Lashley

**A Single cell omics approach to neurodegeneration** P42
*L. Greensmith*

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# Oral presentations

## First scientific session – cerebrovascular disease

### O01

*A.M. Willetts*[1], J. Booker[1], M. Gatherer[1], N.J. Albargothy[1], A. Verma[2], R.O. Weller[1], R.O. Carare[1], C.A. Hawkes[3]
[1]University Of Southampton, Southampton, United Kingdom; [2]United Neuroscience, Dublin, Rep of Ireland; [3]Open University, Milton-Keynes, UK

**Pathways for flow of solutes along walls of cerebral arteries are maintained even in the presence of cerebral amyloid angiopathy**

Deposition of amyloid-beta (Aβ) in the walls of arteries in cerebral amyloid angiopathy (CAA) is due to a failure of clearance of interstitial fluid and solutes from the brain along the walls of cerebral arteries. We have demonstrated previously that soluble Aβ is eliminated along the basement membranes surrounding smooth muscle cells within the walls of cerebral and leptomeningeal arteries, as Intramural Peri-Arterial Drainage (IPAD). Cerebrospinal fluid (CSF) enters the brain along the pial-glial basement membranes as convective influx/glymphatic flow (CIGF). In this study we test the hypothesis that the pattern of IPAD and CIGF can be maintained in the walls of cerebral arteries even in a model of CAA, in which IPAD is blocked in some arteries by endogenous Aβ. Three 12 month old Tg2576 mice with CAA were injected with fluorescent Aβ into the cisterna magna, followed after 30 minutes by injection of fluorescent Aβ of a different colour into the hippocampus and euthanasia 5 minutes later. Results demonstrate that even in the presence of CAA, there is flow along both IPAD and CPGF associated with the walls of cortical and leptomeningeal arteries. Some Aβ injected into the CSF is observed within Aβ plaques and within IPAD, suggesting that CSF enters the parenchyma along pial-glial basement membranes, diffuses through the extracellular spaces and enters basement membranes of capillaries and within the walls of arteries (IPAD). Our results suggest that the proportion of IPAD and CPGF maintained in CAA should be considered when interpreting the pattern of brain-derived biomarkers in the CSF.

### O02

*E. Norton*[1], L. Bridges[2], L. Kenyon[3], M. Esiri[4], D. Bennett[1], A. Hainsworth[1]
[1]Molecular and Clinical Sciences Research Institute, St George's University of London, London, United Kingdom; [2]Department of Cellular Pathology, St George's University Hospitals NHS Foundation Trust, London, United Kingdom; [3]Department of Pathology, Anatomy & Cell Biology, Thomas Jefferson University Hospital, Philadelphia, USA; [4]Department of Neuropathology, Oxford-Radcliffe NHS Trust, Oxford, United Kingdom

**Mural cell depletion and senescence in cerebral small vessel disease: a neuropathology study in older people (aged 80+)**

*Introduction:* Cerebral small vessel disease (cSVD) characterised by fibrotic thickening in deep penetrating arteries, is a major cause of morbidity in older people. Cell senescence is implicated in vascular ageing and age-related organ dysfunction. We tested whether mural cell depletion and cell senescence associate with cSVD, age and sex in older people.
*Methods:* We examined frontal subcortical white matter from older people (mean age 86 years; range 80–96, $n = 60$) with minimal AD pathology. Sections were labelled immunohistochemically for human smooth muscle myosin and H3K9me3 (trimethylated lysine-9 on histone-3) to identify senescence-associated heterochromatin foci (SAHF). Within small penetrating vessels (20–300 μm), we quantified sclerotic index (SI), mural cell number and density (nuclei/μm2), percentage and area fraction (AF%) of SAHF-labelled nuclei within the vessel wall.
*Results:* SAHF-positive mural cells were seen in the small vessels of all older cases. SI was higher ($P < 0.001$) and mural cell number and density lower ($P = 0.001$, $P < 0.001$, respectively) in cSVD cases relative to aged controls. Mural cell number and density negatively correlated with SI ($P = 0.029$, $P < 0.001$). Percentage of SAHF-positive cells and AF% were lower in cSVD cases compared to aged controls ($P = 0.014$,

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$P = 0.016$). AF% negatively correlated with neuropathological cSVD score and SI ($P = 0.013$, $P = 0.002$). Mural cell depletion and senescence did not correlate with age or sex.
*Conclusion:* In an AD-free cohort of older people, mural cells were quantitatively depleted in cSVD. Cell senescence was evident in both fibrotic and normal-appearing small arteries. Senescent nuclei were less common in cSVD compared to age-matched controls. Our data suggests a role of cell senescence in cSVD pathogenesis.

## O03

*M. Goldfinger*[1], B. Tilley[1], M. Sastre[1], S. Gentleman[1]
[1]Imperial College London, London, United Kingdom

**A tale of two tauopathies: a comparison of vasculature changes in ARTAG and chronic traumatic encephalopathy**

*Introduction:* Chronic traumatic encephalopathy (CTE) is a neurodegenerative disorder associated with repetitive head injury, characterised by the deposition of tau in both astrocytes and neurons around the cerebral microvasculature at the depths of sulci. Aging related tau-astrogliopathy (ARTAG) is a tauopathy associated with advanced ageing, characterised by astrocytic tau near blood vessels and areas of fluid interfaces within the brain. The patterns of tau deposition between both disorders appear strikingly similar, with the two often being confused for one another. As the tau pathology is found near blood vessels, we investigated how the gliovascular unit (GVU) is affected in each disorder.
*Materials and Methods:* With access to the brains of 10 boxers diagnosed with CTE and 11 ARTAG cases, we employed immunohistochemistry and tissue clearing techniques (CLARITY), to investigate the vasculature in each case. This included examination of blood-brain barrier and basement membrane integrity and astrocytic and glia limitans morphology.
*Results:* Based on GVU pathology distribution, ARTAG cases fell into focal or widespread pathology subgroups. Comparison between the groups revealed different regional expression of pathologies, with the ARTAG cohort exhibiting more subcortical changes than CTE. In the ARTAG subgroups, we identified increased perivascular expression of GFAP, white matter AQP4 and increased expression of fibrinogen and microglial marker Iba1. Collagen IV immunostaining revealed greater disruption in CTE when compared to both ARTAG subgroups, with arteriole thickening, capillary loss and CADASIL-like changes.
*Conclusion:* The differences in the GVU disruption associated with tau pathology in CTE and ARTAG, suggest that there may be different underlying mechanisms involved in the two disorders.

## O04

*M.R. Frost*[1], A. Sealy[1], M. Gatherer[1], C. Smith[2], J. Attems[3], R.O. Weller[1], R.O. Carare[1]
[1]University Of Southampton, United Kingdom; [2]Edinburgh University, United Kingdom; [3]Newcastle University, United Kingdom

**Loss of adrenergic receptors on cerebral blood vessels may contribute to the accumulation of amyloid-β in cerebral amyloid angiopathy**

Locus coeruleus is the source of adrenergic nerve supply for the brain parenchyma and it degenerates early in Alzheimer's disease (AD). Failure of clearance of fluid, amyloid and other proteins from the brain is a prominent pathological feature of AD. Fluid and solutes are eliminated from the brain parenchyma along the basement membranes of cerebral capillaries and arteries as Intramural Peri-Arterial Drainage (IPAD) and the motive force for this process appears to be derived from the contractions of arterial smooth muscle cells. Here we test the hypothesis that adrenergic receptors are expressed in the walls of both mouse and human blood vessels and the percentage area immunostained for adrenergic receptors is reduced in AD. We used immunocytochemistry for α1A and α2A-adrenergic receptors followed by microscopy in 3 month old C57/Bl6 mice and in occipital tissue from young, old non-demented and in cases of AD with moderate CAA obtained from Edinburgh and Newcastle Brain Banks. Results demonstrate the presence of both α1A and α2A on the walls of capillaries, arteries and veins in mouse and human brains, although α2A was observed mostly on small vessels in the mouse and large vessels in human brains. The percentage area immunostained for both α1A and α2A receptors was similar in young and old human brains but the percentage was decreased in AD. These results suggest that adrenergic input to cerebral blood vessels is reduced in AD and most likely contributes to the failure of IPAD and the deposition of

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amyloid and other proteins in the brain and walls of blood vessels.

## O05

*H. Tayler*[1], R. MacLachlan[1], I. Schulz[1], O. Skrobot[1], S. Miners[1], S. Love[1]
[1]University Of Bristol, Bristol, United Kingdom

**Post-mortem biochemical markers of vascular dysfunction: associations with midlife hypertension, VCING, endothelin-1 and dementia**

Midlife hypertension is a major risk factor for vascular dementia (VaD) and is also associated with Alzheimer's disease (AD). We used biochemical methods to assess white matter perfusion, microvascular content and blood-brain barrier disruption in homogenates of frontal and parietal white matter from a mixed post-mortem cohort of AD ($n = 104$), VaD ($n = 22$), mixed pathology ($n = 35$) and control brains ($n = 57$), and examined the relationship between these markers and (i) mid-life hypertension; (ii) endothelin-1 (EDN1) level; and (iii) vascular cognitive impairment neuropathology guidelines (VCING) score (Skrobot et al, Brain 2016;139:2957).

Ante-mortem perfusion was assessed by determining the ratio of myelin associated glycoprotein (MAG) to proteolipid protein-1 (PLP1) (Barker et al, J Cereb Blood Flow Metab 2013;33:1050), and measuring the concentration of vascular endothelial growth factor-A (VEGF) (Thomas et al, Brain 2015;138:1059). We also measured EDN1; von Willebrand factor (VWF) to indicate microvessel density; the pericyte marker, platelet-derived growth factor receptor-β (PDGFRB); and fibrinogen (FGA) to assess blood-brain barrier leakage.

Midlife blood pressure measurements from clinical records were used to stratify cases into quartiles. The top quartile had increased EDN1 and FGA, and lower MAG:PLP1. EDN1 correlated positively with VEGF and negatively with MAG:PLP1. Intermediate and high VCING scores were associated with significantly elevated VEGF, VWF and PDGFRB, and lower MAG:PLP1. Both VCING score and MAG:PLP1 were highly significant predictors of dementia.

## O06

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**Cerebral amyloid angiopathy in Lewy body dementias**

Lewy body dementias are the second most common neurodegenerative dementia in patients older than 65 years. They are often described as a spectrum of Lewy body disorders where dementia with Lewy bodies (DLB) represents one end nearing AD and Parkinson's disease dementia (PDD) the other end nearing Parkinson's disease (PD). We can clinically differentiate between DLB and PDD by using '1 year rule' which includes the temporal onset of parkinsonism and dementia. However, it can be difficult to differentiate between them on neuropathological level without sufficient clinical information. Therefore, the conundrum whether PDD and DLB represent one or two different disorders remains unresolved.

We hypothesised that PDD and DLB are two different disorders and our aim was to identify a novel neuropathological difference by investigating other co-occuring neuropathologies, such as cerebral amyloid angiopathy (CAA).

Our cohort included 52 PDD and 12 DLB cases. CAA was more frequent ($P = 0.013$) and more severe ($P = 0.010$) in DLB than in PDD. Topographical distribution of CAA showed higher CAA scores in different brain regions (temporal lobe, $P = 0.034$; parietal lobe, $P = 0.045$; occipital lobe, $P = 0.019$ and the cerebellum, $P = 0.027$) as well as significantly higher total CAA score ($P = 0.016$) in DLB compared to PDD. CAA scores in the frontal lobe were similar, $P = 0.188$.

We conclude that CAA with its higher frequency, severity and higher scores in different brain regions in DLB represents a novel neuropathological difference between DLB and PDD.

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# Second scientific session – Alzheimer's disease

## O07

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**Proteomic differences between Alzheimer's disease and frontotemporal dementia human post-mortem brains**

*Introduction:* Alzheimer's disease (AD) is the most common form of dementia and can be sporadic or familial in nature. Frontotemporal dementia is the second most common form of dementia under 65 and can be pathologically divided into multiple subtypes depending on the protein found in the inclusions. The FTLD-TDP cases all consist of TDP-43 pathology, which can be further split into four subtypes based on the type and distribution of inclusions present. Both forms of dementia have similarities and differences and at present there is no biomarker that can properly distinguish the diseases in life. Here we investigate differences in the proteomic profile between sporadic and familial AD cases (SAD and FAD) and different FTLD-TDP subtypes.
*Methods and materials:* Frontal cortex homogenate from post-mortem human brain tissue was prepared from control ($n = 6$), SAD ($n = 7$), FAD ($n = 9$), FTLD-TDP A ($n = 6$), FTLD-TDP A with an expansion repeat in the C9orf72 gene ($n = 6$), FTLD-TDP B ($n = 2$) and FTLD-TDP C ($n = 6$) cases. Proteins were quantitated using 2D-LCMS and UDMSe label- free mass spectrometry.
*Results:* The proteomics showed a range of upregulated and downregulated proteins in all cases when compared to controls. Each dementia had a unique proteomic profile. Changes in expression were observed between proteins involved in neurodegeneration, such as APOE and MAPT between the disease groups. Pathway analysis also highlighted that pathways are altered between different dementias.
*Conclusions:* Investigating the proteins uniquely changing in expression and the pathways which are altered in each of these dementias will help identify disease specific pathogenesis and help to further elucidate dementia mechanisms.

## O08

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**Cholesterol dysregulation in the neurone-astrocyte unit as an early contributor to neuronal dysfunction in Alzheimer's disease**

*Background:* Cholesterol is essential for neuronal function but high cholesterol is a risk factor for Alzheimer's disease (AD). Astrocytes undertake the bulk of cholesterol synthesis; however, under stress conditions neurones can also synthesise cholesterol. We previously showed that a neuronal DNA damage response is associated with lower cognitive function at the earliest stages of AD neuropathology, and this is associated with reduced expression of genes for cholesterol biosynthetic enzymes. We hypothesised that dysregulation of cholesterol metabolism in neurones and astrocytes, contributes to neuronal dysfunction with AD progression. Our initial study focused on the expression of HMG CoA reductase (HMGCR), the rate-limiting enzyme for the cholesterol biosynthesis pathway.
*Methods:* The cellular expression of HMGCR was investigated in the temporal cortex (Brodmann area 21/22) in an ageing population-based sample derived from the Cognitive Function and Ageing Study ($n = 99$). Expression was determined using immunohistochemistry, and quantified by image analysis of the percentage area of immunoreactivity.
*Results:* In the ageing brain, expression of HMGCR was mainly associated with cortical pyramidal neurones, suggesting that, cholesterol synthesis is undertaken by neurones. There was population variation in its expression but no significant association with Alzheimer's type pathology, oxidative DNA damage or neuroinflammation.
*Conclusions:* HMGCR is expressed within neurones; the cause of variation in expression is currently unexplained. Further work will extend these studies to

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assess expression of other enzymes of the cholesterol biosynthesis pathway, both at the protein and gene expression level in whole tissue and neuronal and astrocyte cell populations enriched by laser capture microdissection.

## O09

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**Human transmission of amyloid-β cerebral amyloid angiopathy**

*Introduction:* Experimental seeding of amyloid-β (Aβ), the most common misfolded protein in the ageing brain, has been demonstrated in animal models. We and others have previously shown that Aβ transmission has also occurred through medical procedures in humans, and we show that Aβ proteopathic seeds may also be transmissible through neurosurgery and similar, invasive procedures.
*Material and methods:* The pathology archive at the National Hospital for Neurology and Neurosurgery was searched for brain biopsies of young adults with cerebral amyloid angiopathy (CAA) and appropriate controls. Patients with history of neurosurgery were identified and CAA, extent of parenchymal Aβ and tau pathology, genetic data for mutations (APP, PSEN1, PSEN2 and APOE polymorphism) were determined. In addition, we identified in the literature further patients with young onset CAA.
*Results:* Four patients with histologically confirmed CAA and a past medical history of neurosurgery were identified. Three had undergone diagnostic brain biopsy for investigation of intracerebral haemorrhage and one had died of complications from intracerebral haemorrhage. All four patients (three females and one male) had undergone neurosurgery several decades earlier for brain trauma, developmental malformation, intracranial tumour and syringomyelia. None of the patients had mutations in genes associated with early Aβ pathology. In the literature, we identified four additional patients with CAA and history of childhood neurosurgery.
*Conclusions:* The history of neurosurgical procedures, the absence of known pathogenic mutations and development of CAA-related brain haemorrhages three decades later provides further circumstantial evidence that Aβ proteopathic seeds may have been transmitted by surgical instruments carrying traces of misfolded Aβ protein.

## O10

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**Fibrinogen leakage in the white matter of Alzheimer's disease and normal aged brains: Implications for fibrinogen as a biomarker**

*Background:* The blood-brain barrier (BBB) regulates cerebrovascular permeability and leakage of blood-derived fibrinogen has been associated with cerebral small vessel disease (SVD), white matter lesions (WML) and the pathogenesis of Alzheimer's disease (AD), with the presence of CSF plasma proteins suggested to be a potential biomarker of AD. We determined if extravascular fibrinogen in the white matter was associated with the development of hyperphosphorylated tau (HPτ), amyloid-β (Aβ), SVD and WML.
*Materials and methods:* Parietal tissue from 20 AD and 22 non-demented controls was quantitatively assessed for HPτ, Aβ, WML severity, axonal density, demyelination and extravascular fibrinogen in both WML and normal appearing white matter (NAWM). SVD severity was determined by calculating sclerotic indices.
*Results:* WML- and NAWM fibrinogen burden was not significantly different between AD and controls nor was it associated with the burden of HPτ or Aβ pathology, or any measures of white matter damage.

SVD was associated with and a predictor of both higher WML- and NAWM fibrinogen burden (both $P < 0.05$) in controls only. In cases with minimal SVD NAWM fibrinogen burden was significantly higher in the AD cases ($P < 0.05$).
*Conclusions:* BBB dysfunction was present in both non-demented and AD brains and was not associated with

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the burden of AD-associated cortical pathologies but it was associated with SVD in non-demented controls only. In cases with minimal SVD, BBB dysfunction was significantly worse in AD cases possibly due to CAA. Extravascular fibrinogen is associated with SVD and not AD hallmark-pathologies, indicating its presence in CSF is a marker for cerebrovascular pathology and not AD pathology.

### O11

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**Heterogeneity of cellular inflammatory responses in ageing white matter and relationship to Alzheimer's and small vessel disease pathologies**

*Background:* White matter lesions (WML) are common in ageing, show an independent relationship to cognitive impairment, and are associated with Alzheimer's Disease (AD) and vascular disease (VaD). However, the relative contributions of AD and VaD to age-related WM degeneration are not known. Using the Cognitive Function and Ageing Study (CFAS) neuropathology cohort we hypothesised that variation in the neuroinflammatory response in WML relates to the underlying causes of pathology.
*Methods:* Standard immunohistochemistry (IHC) was employed to investigate astrogliosis (GFAP) and microglial reactivity (Iba1, CD68 and MHCII) in parietal WM. Computer-aided image analysis of immunoreactivity was assessed across the WM, to include subcortical, mid and subventricular regions. Astrocyte morphology was classified as fibrillary or clasmatodendritic (CD). These neuroinflammatory markers were related to vascular and Alzheimer's pathology.
*Results:* There was significant variation in the neuroinflammatory response across the WM regions. Correlation and principal component analyses suggest 1) an immune response grouping (MHCII, Iba1, CD) particularly increasing towards subventricular regions, 2) a CD68+ group related to MRI WML focal lesions. WM pathology showed no correlation with overlying AD pathology, while only the immune group suggested a correlation with markers of vascular pathology (cerebral amyloid angiopathy and microinfarcts).
*Conclusion:* Assessment of neuroinflammatory markers reveals heterogeneity in the cellular pathology of age-related WM degeneration. These patterns vary with location in the WM and may show differential relationships to putative pathological causes of WM degeneration.

## Third scientific session – Neurodegeneration

### O12

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**Profiling of tauopathies in frontotemporal dementia**

Around 40% of patients with frontotemporal lobar degeneration (FTLD) have tau-positive inclusions at post-mortem with unique tau confirmations representing specific FTLD-tauopathies. Currently there are no reliable biomarkers that can detect during life specific tauopathies and differentiate them from non-tau pathologies. In this study we profiled FTLD cases using

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antibodies targeting different regions and conditions of the protein tau, with the aim of identifying different exposed epitopes which could lead to in-life diagnosis of the specific pathologies.

All cases were provided by Queen Square Brain Bank, UK. Immunohistochemical staining with twelve anti-tau antibodies spanning the length of the protein and different phosphorylation states was performed on temporal and frontal lobes as well as the hippocampus of post-mortem cases. These included five cases from four FTLD-tau subtypes (Pick's disease, progressive supranuclear palsy, corticobasal degeneration and globular glial tauopathy) and Alzheimer's disease cases used as a control.

Previously identified pathological hallmarks known to be present in the different tauopathies were positively stained with many of the epitope specific tau antibodies. Antibodies recognising the C-terminus of the tau protein stained the majority of AT8 positive tau inclusions. Whereas there was more variation in the N-terminal specific antibodies.

These findings identify disease specific tau fragments that could be utilised as novel biomarkers for the diagnosis of tauopathies cases during life.

## O13

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**Quantification of neurons in the inferior frontal gyrus in frontotemporal lobar degeneration**

In this study we aimed to quantify the laminar specificity of cell loss in two types of frontotemporal lobar degeneration, namely progressive supranuclear palsy (PSP) and the behavioral variant of frontotemporal dementia (bvFTD). Despite differences in the classical clinical and neuropathological presentation of bvFTD and PSP, cognitive and behavioral changes associated with the prefrontal cortex such as apathy and impulsivity are common in both diseases. We used stereology to quantify the total number of neurons in the inferior frontal gyrus (IFG) in cases with bvFTD ($n = 6$), PSP ($n = 8$) and age matched controls ($n = 7$). The entire left IFG was dissected from formalin fixed brains, paraffin embedded and sectioned coronally in serial sets of 40 μm sections, yielding 15–25 sections per series. Sections were stained with Giemsa and neurons were counted using the StereoInvestigator Software. Interim analysis showed a 42% loss of neurons in superficial layers (56 million bvFTD vs 89 million Control, $P = 0.047$) and 44% loss of neurons in deep layers (45 million bvFTD vs 71 million Control, $P = 0.024$). In addition there was a significant volume loss of 50% ($P = 0.003$) in superficial and 49% in deep layers (TukeyHSD $P = 0.008$). There was no neuronal loss in PSP in the superficial (88 million, $P = 0.99$) or deep cortical layers (68 million, $P = 0.89$) compared to controls. Nor did we find a significant loss of volume ($P$, superficial = 0.55, p, deep = 0.54). Thus, behavioral symptoms in PSP relating to the IFG may be caused by functional or connectivity impairment rather than cell loss, e.g. synaptic loss, reduced plasticity, and metabolic impairment.

## O14

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**The role of tau and basal ganglia cholinergic pathology in the pathogenesis of Parkinson's disease motor subtypes**

*Introduction:* Parkinson's disease (PD) is a progressive neurodegenerative disorder with two main motor presentations: tremor-dominant (TD) or akinetic-rigid (AR). We have shown in previous studies that substantia nigra (SN) pathology does not differentiate these subtypes. SN neurons modulate the activity of the highly-arborised striatal cholinergic interneurons (ChIs). ChIs are dysregulated in PD and there is evidence of differential cholinergic tone in motor subtypes. The present study aimed to assess striatal pathology, especially in ChIs in PD motor subtypes.
*Methods:* 40 TD and 40 AR-presenting cases were selected from the Parkinson's UK Tissue Bank. Tissue sections from the basal ganglia, at the anterior commissure level, were immunostained for α-synuclein, AT8, 3R and 4R tau, amyloid-β and choline acetyltransferase (ChAT). ChI arborisation was assessed by counting primary and secondary branches. Pathology was quantified using a percentage-coverage methodology.

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*Results:* A significant reduction in primary and secondary branching was seen in ChIs of AR cases compared to TD. Striatal α-synuclein and tau pathology was significantly increased in AR cases. AR cases also displayed AT8 tau-positive tangle pathology in the striatum that co-localised with ChAT. These structures were both 3R and 4R-tau positive.
*Conclusions:* This study is the first to investigate striatal pathology as a means of differentiating PD subtypes. Tau pathology of ChIs in the striatum, a newly described phenomenon, was more common in AR cases than TD. AR PD was characterised by 3R and 4R-tau-positive, atrophic ChIs, reflecting a novel hypothesis with a role for tau in the pathogenesis of differential motor presentations in PD.

## O15

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**Investigation of alpha-synuclein post-translational modifications in idiopathic Parkinson's disease and multiple system atrophy**

*Introduction:* Aggregated alpha-synuclein (a-syn) is a key component of Lewy bodies (LBs) and Lewy neurites (LNs) which are the defining pathological hallmarks of Parkinson's disease (PD). The pathological filamentous oligodendroglial inclusions in multiple system atrophy (MSA) also contain aggregated a-syn. A-syn can exist in several forms from monomers to oligomers to fibrils and can also be post-translationally modified including nitration and phosphorylation. Herein we aimed to investigate the different a-syn species in PD and MSA cellular inclusions.
*Material and methods:* Formalin fixed human brain tissue from 15 PD, 5 MSA and 5 neurologically normal controls were obtained from the archives of Queen Square Brain Bank. Using routine protocols, immunohistochemistry was performed with 4 a-syn antibodies, three of which were specific for different post-translational modifications namely, phosphorylation (Ser129 and Ser87-residues) and nitration (Tyr39-residue) in 8 different brain regions.
*Results:* All the a-syn antibodies recognised LBs and LNs in PD. Phospho-Ser129 and nitrated a-syn antibodies also highlighted thin neurites and dot-like structures. Phospho-Ser87a-syn was present in fewer LBs and LNs. Glial cytoplasmic inclusions (GCIs) were the dominant pathological structure recognised in MSA cases.
*Conclusion:* Both nitrated and phosphorylated forms of a-syn are present in pathological inclusions in PD and MSA. Phospho-Ser129a-syn highlights thin LNs and dot –like structures in addition to classical Lewy bodies. This may indicate differential phosphorylation of a-syn in different pathological inclusions in PD.

## O16

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**DNA methylation landscapes in patients with multiple system atrophy**

*Introduction:* Multiple system atrophy (MSA) is a fatal late onset neurodegenerative disease. MSA is mostly sporadic and its aetiology remains elusive. Clinically, it is characterized by a variable combination of parkinsonism, ataxia and autonomic failure. Due to its clinical presentation overlapping with other neurodegenerative disorders, including Parkinson's disease, a definite MSA diagnosis can only be confirmed at post-mortem. The presence of α-synuclein within oligodendrocytes in the form of glial cytoplasmic inclusions (GCIs) is the diagnostic hallmark of MSA. Pathologically, MSA can be categorised into striatonigral degeneration, olivopontocerebellar atrophy or mixed subtypes. To get insights into molecular mechanisms

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associated with MSA, we have investigated DNA methylation profiles.
*Methods:* We have profiled DNA methylation patterns in brain samples of MSA cases (mixed subtype) and controls. We have collected three brain regions per individual: the cerebellum, and the frontal and occipital cortices. To enrich for glial cells, where the GCIs are found in MSA, we have selected white matter tissue. We have investigated global DNA methylation changes by immunohistochemistry as well as changes at single nucleotide resolution by using Illumina Infinium MethylationEPIC BeadChip arrays, which evaluate over 850,000 methylation sites per sample.
*Results:* While global levels of DNA methylation did not differ significantly between cases and controls, preliminary results from the EPIC arrays identified important loci with significantly altered DNA methylation levels between cases and controls. We will present these loci and discuss their relevance to the disease process.
*Conclusion:* Our data suggests that changes in DNA methylation patterns at specific loci are associated with MSA.

## O17

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**DNA methylation in amyotrophic lateral sclerosis (ALS)**

*Introduction and rationale:* ALS can be sporadic (sALS) or familial, with a number genes implicated including C9orf72 (C9ALS) and TARDBP in the latter. DNA methylation is an epigenetic mechanism whereby a methyl group is attached to a cytosine, usually resulting in gene expression repression. DNA methylation has been implicated in other neurodegenerative diseases, but little work has been conducted in ALS. We aim to elucidate the role of DNA methylation in motor neurone (MN) decline, without the interactions from other cell types, which may mask MN-specific DNA methylation changes.
*Methods:* Immunohistochemistry (IHC) was used to determine the pathology of 5mC, TDP43 and 5hmC within cervical spinal cord. From a subset of the same cohort, MNs were extracted from the anterior horn using laser capture microdissection (LCM). DNA was then extracted, analysed using the Infinium HD Methylation assay, and analysed using RnBeads.
*Results:* Immunohistochemistry revealed increased methylation in ALS, with C9ALS displaying the highest global methylation. Interestingly, methylation levels appeared to reduce in the minority of cells that showed loss of nuclear TDP43. Microarray data also showed significantly increased methylation for ALS cases when compared to controls, with PANTHER pathway analysis implicating many known neurodegenerative disease related pathways.
*Conclusions:* DNA methylation is a contributory factor in ALS, with our data suggesting hypermethylation in particular, is involved in ALS. Further study into the genes and promoters identified could help to elucidate biomarkers for ALS in the future.

## O18

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**UK Brain Banks Network Database: a resource for researchers and brain banks**

The UK Brain Banks Network (UKBBN) is a coordinated national network of 10 UK brain banks that operate to uniform standards and share data on over 17,000 brains, over 11,000 of which are accessible to researchers in academia and industry worldwide. The Network supports a wide range of research into causes, manifestations, diagnosis and effects of treatment of diseases of the CNS. The participating brain banks all have their own research ethics approval to function as research tissue biobanks. Data are securely uploaded to the UKBBN database from the brain banks and from other databases such as the Brains for Dementia Research Clinical Assessment Database. A web-based interface (https://brainbanknetwork.cse.bris.ac.uk/) allows researchers to access clinical and neuropathological data on brains from across the Network, through an interface that allows combination of multiple search parameters. The UKBBN database is also used to log research studies and tissue requests and to link to a sample tracking system (CSols RFTrackIT™). These facilities enable administrators within each brain bank to use the database to manage tissue requests

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and approvals, monitor sample availability and usage, and readily compile data for funders, the HTA and research ethics committees.

Advances in the diagnosis, understanding and treatment of human neurological disease have depended on analysis of well-characterised human brain tissue curated by brain banks. In the UK this is now facilitated by the UKBBN database, use of which has increased steadily. Currently the database has over 600 active users in 24 countries, and hosts over 680 sessions each month, about 1/3 from users outside of the UK.

# Fourth scientific session – Brain Tumours

## O19

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**Characterisation of gliomas with rare IDH mutation at QEHB during 2016–2018**

*Introduction:* Mutations in isocitrate dehydrogenase-1 or -2 (IDH1 or IDH2) are found in most of WHO grade II / III diffuse gliomas comprising oligodendrogliomas (~70–85% IDH mutant) and astrocytomas (~65–80% IDH mutant). While the vast majority of IDH mutations represent IDH1 R132H (~88%), the remaining 12% of rare variants of IDH1 and IDH2 mutations have not been comprehensively analysed. We correlate these with histological and clinical features.

*Results:* Among 204 glioma samples analysed for IDH mutations, 26 rare IDH1 and IDH2 mutations were identified. 20 out of 26 (76%) had a location in the frontal lobe. There were 20 rare IDH1 mutations and 6 IDH2 mutations. Our local methodology allowed categorising the rare IDH1 mutations into two groups, the IDH1 non-R132H/C group (50%) and IDH1 R132C group (27%), while IDH2 mutants were grouped into IDH2 R172K (15%) and IDH2 non-R172K (8%). Among the non-R132H/C group, 8 (61%) were astrocytic tumours, and 5 (38%) were oligodendroglial tumours. All the IDH1 R132C variants were astrocytic tumours. Similarly, all IDH2 non-R172K were astrocytomas. All the IDH2 R172K variants were oligodendrogliomas with 1p19q co-deletion. ATRX and p53 mutations were highest in the IDH1 R132C group, followed by IDH1 non-R132H/C group, while IDH2 non-R172K also showed these mutations. Interestingly, MGMT methylation was absent in 7 out of 26 cases (26%), and low in 5 (19%).

*Conclusions:* The majority of rare IDH1 mutations were found in astrocytic tumours, while IDH2 mutations were frequent in oligodendrogliomas, although specific variants showed higher association with different phenotypes. Many rare IDH mutants had unmethylated MGMT which has therapeutic implications.

## O20

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**Paediatric brainstem glioma and its intersection with brain development: role of tumour microenvironment**

Diffuse intrinsic pontine glioma (DIPG) is a rare aggressive childhood malignancy with no cure and children seldom survive 2 years after diagnosis. Whilst there is extensive research to determine the function of molecular aberrations in DIPG, there are significant gaps in understanding the influence of tumour microenvironment and brainstem development. A multi-methodological approach to profile the DIPG/host landscape in the context of developing brainstem could provide information to inform work in identifying potential therapeutic targets. Bioinformatic analyses using the R2: Genomics Platform was conducted. In parallel; fixed-formalin paraffin embedded DIPG tissue was obtained from post-mortem brain for focused RNA arrays and RNAseq. RNA was extracted from tumour core, adjacent and 'normal appearing' tissue. Following quality control experiments to ensure RNA integrity, RT-PCR array and RNAseq experiments were conducted. 'Proof-of-principle' experiments entailed the examination of 80 genes selected for the extracellular matrix (ECM) focused array. Analyses of a publically

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available DIPG microarray data showed that 15/80 ECM related genes were significantly higher in DIPG tumours compared to normal brainstem ($P < 0.05$). In RT-PCR array, 7/80 genes were elevated in tumour core. 4 genes overlapping between bioinformatic and RT-PCR data were further analysed using R2 platform (Brainspan.org) of normal brain development to help separate expression associated with development from tumour. Focusing on the 4 overlapping genes RNAseq analyses showed that TNC and COL11A1 expression was increased 2 fold in tumour. Results from protein validation immunohistochemistry will be presented. By using a multi-pronged screening we provide a 'proof-of-principle' approach whereby genes not previously characterised in DIPG were identified to be differentially expressed.

## O21

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**The biology of paediatric brain tumours at post-mortem: a national cohort**

*Background:* Central nervous system (CNS) tumours are the leading cause of childhood cancer deaths. At the points when tumours become untreatable, our understanding of their pathogenesis is limited but studies suggest the biology of tumours at relapse and death differ from presentation. We have assembled a post-mortem paediatric brain tumour cohort and assessed the feasibility of using archival post-mortem tissue with modern genomic technologies.
*Methods:* Cases were identified through BRAIN UK and local databases. Clinico-pathological data was analysed for the local cases and a subset underwent DNA methylation array profiling. Methylation data was processed with the DKFZ CNS tumour classifier and conumee package.
*Results:* We identified over 200 post-mortem paediatric CNS tumours. Among local cases, we observed variations in age; presenting symptoms; treatment; survival time until death; tumour type; tumour origin and infiltration. High-grade glioma was the most common tumour type, although some cases have unresolved diagnoses. Infiltration to at least one area outside the primary tumour site was commonly observed. In a proportion of cases, cause of death was not due to the brain tumour but due to another disease, most commonly bronchopneumonia.

Using the DKFZ model to process methylation data, the proportion of classified post-mortem cases was comparable to a clinical cohort of surgical cases. A proportion of cases analysed had interpretable copy number plots.
*Conclusions:* We have established a post-mortem paediatric CNS tumour cohort and demonstrated that post-mortem brain tissue is suitable for DNA methylation profiling. The cohort is heterogeneous but by focusing on subgroups, molecular analyses may increase our understanding of why brain tumours become untreatable.

## O22

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**The cancer stem cell marker ALDH1A1 is upregulated and drives proliferation in merlin null meningioma and schwannoma**

*Background:* Treatment options for the brain tumours schwannoma and meningioma are limited to surgery and radiotherapy. Merlin (NF2) is a tumour suppressor which is deleted in approximately 60% of meningiomas and schwannomas. Loss of NF2 increases the transcriptional activity of the Hippo pathway effectors YAP and TAZ, and we now find leads to increased expression of cancer stem cell markers in these tumours.
*Methods:* A mouse RNA sequencing screen was conducted in which NF2 was removed from Schwann cells

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specifically to identify differentially regulated genes which may be involved in tumour initiation and growth. Validation of cancer stem cell markers was completed in primary human meningioma and schwannoma tumour material.
*Results:* RNA sequencing identified that the cancer stem cell marker aldehyde dehydrogenase 1A1 (ALDH1A1) was significantly upregulated in nf2 null mouse sciatic nerve both pre and post-injury. Aldh1a1 upregulation is almost exclusive to the non-myelinating Schwann cells in intact nerves. Loss of TAZ in NF2 null sciatic nerve returns aldh1a1 expression to control levels. ALDH1A1 was also expressed in human Merlin null primary human meningiomas and schwannomas, lentiviral mediated knockdown of TAZ reduced expression of ALDH1A1. Inhibition of ALDH1A1 using novel specific inhibitors significantly reduced proliferation in primary meningioma and schwannoma cells.
*Conclusions:* The cancer stem cell marker ALDH1A1 is upregulated in a TAZ dependent manner following loss of Merlin in mouse Schwann cells and human meningioma and schwannoma. Understanding Aldh1a1-dependent signalling may lead to new potential therapies for these clinically important tumours.

### O23

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**Intracerebral lymphoid malignancies: a retrospective study of morphological, immunohistochemical and molecular characteristics with survival correlation**

*Background:* Intracerebral lymphoid malignancies are rare. PCNSLs constitute the majority of cases and most are of ABC subtype. MYD88 L265P mutation is one of the most frequently recurring molecular abnormalities in PCNSL. In this study, we aimed at comprehensive characterisation of intracerebral lymphoid entities by integration of pathological, molecular and survival data.
*Methods:* We retrospectively reviewed the pathology and immunohistochemistry of sixty cases of intracerebral lymphomas diagnosed over an 11-year period in a major diagnostic unit in Scotland. Cases were classified using the 2017 WHO classification and were tested for MYD88 mutation by PCR. Correlations were made between WHO defined pathological entities, molecular results and survival data.
*Results:* PCNSL constituted 82% of cases and systemic DLBCL 5% of cases. All except one were of ABC subtype. MYD88 L265P mutation was tested in 53% of cases, of which 75% were mutated and 25% were wild type. Mutated MYD88 was found in 81% of tested PCNSLs. The presence of MYD88 mutation did not predict a poor prognosis.
*Conclusions:* Our results demonstrate that ABC subtype of PCNSL is the commonest intracerebral lymphoid malignancy. MYD88 mutation is present in a high proportion of PCNSLs, and, in our study, it was not associated with poor survival.

## Fifth scientific session – Neuroinflammation

### O24

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**Extracellular matrix proteins interface with HLA genotype to modulate topographical variation of multiple sclerosis pathology**

Multiple sclerosis (MS) is typified by its clinical and pathological heterogeneity, classically diagnosed based

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on its 'dissemination in time and space'. The topographical variation of pathology down the neuraxis in MS remains an understudied and underappreciated area, despite it being one of the core aspects of the disease. In particular, the extent of inflammation and demyelination varies substantially within the CNS, being most severe in the cervical spinal cord. Any mechanical or anatomical influence at play likely interfaces with immunopathological mechanisms, since HLA-DRB1*15 status exaggerates topographical differences in the spinal cord and influences the relationship between inflammation, demyelination, and axonal loss. Here, we used shotgun proteomics to compare protein expression in spinal cords of HLA-DRB1*15-positive ($n$ = 3) and -negative ($n$ = 2) MS cases. The 3 top differentially expressed proteins, found in higher levels in DRB1*15-positive cases, were the extracellular matrix proteins biglycan, decorin and proline/argine-rich end leucine-rich repeating protein (PRELP). We subsequently undertook neuropathological analysis using immunohistochemistry to compare distribution of these proteins down the neuraxis in the cortex, cervical and lumbar spinal cords of post-mortem MS tissue ($n$ = 47) and non-neurological controls ($n$ = 7). We highlight striking topographical differences in perivascular and parenchymal distributions of these proteins, comparing MS cases vs controls, and HLA- DRB1*15-positive ($n$ = 21) and -negative ($n$ = 26) cases. We compare these protein levels with markers of inflammation and neuronal/axonal density, since all 3 proteins are known to interface with the immune system. Overall, this work demonstrates the power of applying non-biased proteomics using human post-mortem tissue to identify novel protein candidates that potentially influence the topographical variation of MS disease processes.

## O25

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**The role of immunity in human motor neuron disease (MND)**

Motor Neuron Disease (MND) is a fatal, neurodegenerative condition characterised by progressive motor neuron loss. The average survival time is only two-three years; however, this varies greatly. It is becoming clear that glial and immune cells actively contribute to disease progression. Microglia, the primary immune cells of the CNS, have both a neuroprotective and neurotoxic effect in most neurodegenerative diseases, and are highly associated with MND pathology. However, their role in MND is unclear. We aim to elucidate the role of immunity in MND by examining the key immune gene expression pathways in disease, and those gene expression changes that correlate with variability in patient survival time.

RNA was extracted from the ventral horn of formalin fixed paraffin embedded (FFPE) spinal cord samples from MND patients with varying survival times, and neurologically healthy controls. RNA was of sufficient quality and quantity to perform gene expression using the NanoString Gene Expression Assay – Inflammation Profile and analysis was carried out using the nSolver package.

Comparison of the normalised gene expression values from the MND and control group has highlighted 106 upregulated genes with a fold change greater than 1.5. Gene expression analysis of the MND cases alone, revealed 90 genes, which are upregulated proportionally to patient survival time. nSolver pathway analysis implicated many known neurodegenerative pathways including the PI3K-AKT and NF-Kappa B pathways.

Immunity gene expression pathways differ between control and MND tissues and correlate positively with greater survival times. Further research will aim to validate these changes using immunohistochemistry on tissue microarrays, to highlight candidate pathways for disease modifying treatments.

## O26

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**The structural basis of Susac's syndrome**

*Background:* Susac's syndrome (SS) is characterized by the clinical triad of encephalopathy, hearing loss and branch retinal artery occlusion. An underlying autoimmune microangiopathic process has been suggested based on the presence of circulating anti-endothelial cell antibodies (AECAs) and endothelial staining for complement components (C3d and C4d). No brain autopsies have yet been described.

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*Case:* We present a 47-year-old female with encephalopathic SS. She had a 10-year history of neuropsychological problems including episodes of confusion, aphasia, psychosis, depression, migrainous headaches and seizures. Protein levels in the cerebrospinal fluid were mildly elevated. Serial neuroimaging showed progressive cerebral atrophy and numerous small hypodense areas appearing as central "holes" in the corpus callosum. Autopsy showed generalized brain atrophy and thinning of the corpus callosum. Microscopy revealed widespread proliferation of the capillaries accompanied by endothelial cell loss, thickened hyalinized walls, and patchy labeling of blood vessels by antibodies to membrane attack complex (MAC). Innumerable vessel-centered small patches with loss of cell bodies (neurons and oligodendrocytes) and demyelination were scattered in the white and gray matter throughout the brain and spinal cord. The lesions appeared as demyelinating due to axonal sparing and absence of cavitation. There were no inflammatory cell infiltrates. Calcifications involving the neuron cell bodies, neurites, and capillaries were noted along with large solid calcified foci predominantly in the pons. Immunostains for SV40, tau, beta amyloid, alpha synuclein, P62 and TDP-43 were negative. Whole-exome sequencing did not reveal any genetic abnormalities.
*Conclusion:* In this first brain autopsy report in SS, autoimmune damage to microvessels is accompanied by innumerable "incomplete" microinfarcts and calcifications.

## O27

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**Choroid plexus acts as gatekeeper for TREM2, abnormal accumulation of ApoE and fibrillary tau in Alzheimer's disease and Down syndrome**

*Background:* Genetic factors that influence AD risk include mutations in TREM2 and allelic variants of Apolipoprotein-E, which are likely to affect AD pathology and in Down syndrome (DS). Evidence shows that dysfunction of the choroid plexus (CP) may compromise the blood-CSF barrier, altering secretary, transport and immune function that can affect AD pathology.
*Objective:* To investigate the genotype and phenotype of DS individuals in relation to choroid plexus damage and blood-CSF barrier leakage to identify markers that could allow early diagnosis of AD in DS.
*Methods:* To assess allele frequency and haplotype associations, ApoE, Tau, TREM2 and HLA-DR were analysed by SNP analysis in DS participants ($n = 47$) and controls ($n = 50$). The corresponding plasma protein levels were measured by ELISA. Post-mortem brains from DS, AD and age matched controls were analysed by immunohistochemistry.
*Results:* Haplotype analysis showed that individuals with Tau H1/H1 and ApoE ε4 genotypes were more prevalent among DS subjects (17%) with an earlier diagnosis of dementia compared to H1/H2 haplotypes (6%). Serum TREM2 levels decreased whereas phospho-Tau levels increased with age in DS. In AD and DS brain insoluble Tau and ApoE was found to accumulate in fenestrated capillaries and TREM2 visible in stromal macrophages in choroid plexus (CP)
*Conclusion:* Accumulation of Tau and ApoE in CP increases oligomerisation of Aβ42 and impair Tau trafficking ultimately leading to AD pathology. We have identified a high-risk haplotype: ApoE ε4, Tau/ H1 and TREM2/T, who has manifest age-related changes that could open a window for treatment, many years prior to the manifestation of the dementia.

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# Sixth scientific session–developmental neuropathology, epilepsy and skeletal muscle

## O28

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**Amygdala development in patients with hippocampal atrophy: associations with socioemotional status**

Lesion-induced plasticity, characterized by neuroblast migration and neuron number increase, has been reported in the amygdala of monkeys with selective neonatal lesion of the hippocampus. In humans, a large number of diseases can lead to hippocampal atrophy but the impact of such damage on the nearby amygdala has not been investigated yet. Here we have used 1.5T MRI acquisitions to study amygdala structure in 25 patients with developmental amnesia (DA), a condition associated with early-life hypoxia-induced bilateral hippocampal atrophy and specific episodic memory impairment; 23 patients with "moderate" hippocampal atrophy; and 36 controls. Our estimates revealed that the amygdala volumes did not correlate with hippocampal volumes in any groups. The amygdala was larger in controls older than sixteen than in younger controls. However there were no such age differences in DA where the amygdala was already enlarged in young patients. An age-dependent increase in amygdala signal intensity was observed in MRI scans of controls while there were no such age differences in DA. MRI findings were analyzed in relation to scores obtained on a parental questionnaire (Child Behavior Checklist). The increased socioemotional and/or behavioural problems in the domains of social, thought, and attention, in patients relative to controls, were significantly correlated with hippocampal but not amygdala volume. Potential abnormal early amygdala development in DA could not account for these symptoms that more likely derived from the patients' memory problems and could be attributable to the direct consequences of the early hippocampal damage and the abnormal interaction of the damaged hippocampus with other brain regions during development.

## O29

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**How does the human neocortex fold? A novel role of the extracellular matrix**

The human neocortex is the seat of many of the higher cognitive functions that make us unique, such as our advanced learning and speech. Yet, in spite of its functional importance, the exact mechanisms driving the development and morphogenesis of the cerebral cortex, and its dysregulation in a wide range of neurodevelopment disorders, remain elusive. This is especially true for several key aspects, such as cortical folding, that remain understudied due to a lack of suitable in vitro systems.

There are several current theories of how the neocortex folds, mainly focusing on genetic (progenitor cell types) or mechanical factors (differential growth of grey vs. white matter). Our recent work suggests that both genetic and mechanical factors are important, and provides a link between the two; the extracellular matrix (ECM).

We focused on three specific ECM components localised in the human fetal cortical plate (11–38 GW): HAPLN1, lumican and collagen-I. Addition of these ECM components to cultures of human fetal neocortex (11–22 GW) caused local changes in ECM stiffness and induced folding of the cortical plate. This ECM-induced folding required an increase in hyaluronic acid, its receptor CD168, and downstream ERK signalling. Importantly, the ECM-induced folds replicated aspects of physiological, nascent folds present at 22 GW, and

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loss of hyaluronic acid reduced both the ECM-induced folds and physiological folds. Finally, this ECM-induced folding was also altered in samples with neurodevelopmental disorders, such as Down syndrome, indicating that the ECM-induced folding assay we developed can now be used as a novel system to study folding disorders and defects in the human neocortex.

## O30

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**Quantitative flow cytometry in the diagnosis of Collagen VI-related disorders: 3-year experience from a tertiary referral centre**

Collagen VI related disorders (COL6-RD) are caused by pathogenic variants in the COL6A1, COL6A2 or COL6A3 genes coding for the three α-chains of collagen VI, resulting in its deficiency or dysfunction in the myomatrix. The associated phenotypes range from the severe Ullrich congenital muscular dystrophy to the milder Bethlem myopathy, with intermediate phenotypes in between. We have previously shown that collagen VI flow cytometry (FC) for quantitative assessment of collagen VI expression in skin-derived fibroblasts (SF) can complement existing immunohistochemical assays in muscle biopsies. However, the diagnostic efficacy of FC, in particular for milder forms of COL6-RD, remains to be established. In this study we evaluated FC in 32 patients referred to our national diagnostic centre for FC in view of suspicion of COL6-RD. FC was performed on cultured SF and collagen VI expression was quantified by mean fluorescent intensity (MFI). MFI reduction >15% over unaffected controls was considered abnormal. Clinical, muscle pathology, muscle MRI and genetic findings were correlated to FC outcome. The FC test positive predictive value was 46% and negative predictive value was 84%. The sensitivity was 66% and specificity was 69%, indicating need for caution in interpreting abnormal MFI values particularly in patients with no supporting clinical, pathological and/or MRI findings. We also present transcript analysis of 4 patients with COL6A1 variants with discordant FC results. Our results indicate that FC can complement diagnosis and aid interpretation of genetic variants in COL6-RD, but analysis of a larger patient cohort with milder forms of COL6-RD is needed to clarify the most reliable MFI threshold for diagnostic purposes.

## O32

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**A novel multiplex chromogenic immunoassay for evaluating mitochondrial respiratory chain complex I and complex IV defects in diagnostic muscle biopsies**

The investigation of clinically suspected mitochondrial disease (mtD) includes performing a muscle biopsy for biochemical/histochemical assessment of mitochondrial respiratory chain (RC) defects. The COX-SDH histochemical assay detects RC-complex IV (CIV) defects, but RC-complex-I (CI) defects cannot be detected histochemically. CI/CIV defects are common in mtD. Immunohistochemical evaluation of RC-complex defects relies on reduced amount of the assembled complex associated with catalytic deficiency, detectable with specific monoclonal RC subunit antibodies. Our aim was to design a brightfield multichromogenic immunoassay for evaluating CI/CIV defects in diagnostic paediatric muscle biopsies. In the dual chromogenic-immunoassay (DCI) optimised protocol, both Abcam primary antibodies were co-incubated (TOMM20-NDUFB8 and TOMM20-MTCO1), and then TOMM20 developed to yellow and the other marker to

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teal (Discovery/Ventana Systems) with regions of colocalisation visualising as green. The DCI and COX-SDH assays were performed in serial frozen sections. 21 biopsies referred to our centre were assessed: 14 with genetically confirmed mtD (mtDNA rearrangements, point mutations and depletion), 4 with high clinical/histological suspicion of mtD, and 3 unaffected controls. % COX and CI/CIV-deficient fibres were ascertained in two random fascicles, with high-level concordance amongst % COX-negative and CI/CIV-deficient fibres. In a proportion of cases the DCI detected more CI-deficient fibres (7/18) and CIV-deficient fibres (5/18) compared to COX-negative fibres (average 6%). Segmental/transitional myofibre CI/CIV defects were detectable. In conclusion, our multiplex DCI reliably detects CI/CIV defects with comparable sensitivity to the conventional COX-SDH assay and can be easily co-opted to routine diagnostic work. Work is underway to develop a quadruple chromogenic immunoassay for digital evaluation of CI/CIV defects.

## O33

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### Cellular expression of SCN1A mRNAs in patients with temporal lobe epilepsy

*Introduction:* Mutations in SCN1A have been reported in various epilepsies, including Dravet Syndromes, and Generalised Epilepsy with Febrile Seizures. In Temporal Lobe Epilepsy (TLE), two single nucleotide polymorphisms in SCN1A (rs7587026; C->A; rs11692675; A->G) are more prevalent in patients with hippocampal sclerosis (HS) and a history of febrile seizures. It is unknown whether there is an association between these allelic variants and mRNA expression of SCN1A in the brains of patients with TLE + HS.
*Material and methods:* Formalin-fixed, paraffin-embedded hippocampi of 14 surgical patients with TLE + HS and rs7587026 CC ($n = 5$), CA ($n = 4$), or AA ($n = 5$) were studied using fluorescent in situ hybridisation and probes against SCN1A transcript variant 1. Labelled sections were scanned using fluorescent whole slide scanner, AxioScan.Z1. Automated quantification of positive labelling was performed using Definiens Developer X64.
*Results:* Positive labelling appeared as small fluorescent puncta, where one punctum represents a copy of SCN1A transcript. In all cases, fluorescent puncta were observed mainly in neurones compared to the neuropils (U-test, $P = 0.000$). The density of fluorescent puncta was significantly higher in the granule cell layer compared to cornu Ammonis and subiculum (Kruskal-Wallis test, $P = 0.002$). The percentage of positive labelling associated with the cell nuclei was significantly different between genotype groups (Kruskal-Wallis test, $P = 0.001$), suggesting a potential association between rs7587026 and mRNA expression of SCN1A in patients with TLE + HS.

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# 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.

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## 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.

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# 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.

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## 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.

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### 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

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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

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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.

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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.

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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

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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.

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## 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.

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## 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.

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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

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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

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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.

## P33

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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.

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## P34

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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

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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.

[1] Walker, L., et al. Acta Neuropathol, 2015. 129 (5): p. 729-48.

## P35

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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.

## P36

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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

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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.

## P37

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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.

## P38

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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

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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.

## P39

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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.

## P40

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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)

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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.

## P41

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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.

## P42

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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

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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.

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