TITLE PAGE

Title: Pathology of neurodegenerative disease for the general neurologist

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ABSTRACT

Neurodegeneration refers to progressive dysfunction or loss of selectively vulnerable neurons from brain and spinal cord regions. Despite important advances in fluid and imaging biomarkers, the definitive diagnosis of most neurodegenerative diseases still relies on neuropathological examination. Not only has careful clinicopathological correlation shaped current clinical diagnostic criteria and informed our understanding of the natural history of neurodegenerative diseases, but it has also identified conditions with important public health implications including variant Creutzfeldt-Jacob disease, iatrogenic amyloid-β and chronic traumatic encephalopathy. Neuropathological examination may also point to previously unsuspected genetic diagnoses with potential implications for living relatives. Moreover, detailed neuropathological assessment is crucial for research studies that rely on curated postmortem tissue to investigate the molecular mechanisms responsible for neurodegeneration and for biomarker discovery and validation. This review aims to elucidate the hallmark pathological features of neurodegenerative diseases commonly seen in general neurology clinics such as Alzheimer's disease and Parkinson's disease, rare but well-known diseases, including progressive supranuclear palsy, corticobasal degeneration, and multiple system atrophy, and more

recently described entities such as chronic traumatic encephalopathy and age-related tau astrogliopathy.

INTRODUCTION:

Most adult-onset neurodegenerative diseases are considered proteinopathies because they are characterised by misfolding of native peptides and proteins such as amyloid-β (Aβ) peptide, tau, α-synuclein, TDP-43 and prion proteins, which then assemble into larger filaments before aggregating to form morphologically distinct cellular inclusions or extracellular parenchymal plaques (figure 1). More than 50 diseases are associated with misfolded protein pathology[1] including well-established clinicopathological conditions such as Alzheimer's diseases (AD) and Parkinson's disease (PD), as well as more recently identified neuropathological entities of uncertain clinical significance such as age-related tau astrogliopathy (ARTAG) and limbic-predominant age-related Transactive DNA-binding Protein 43 (TDP43) encephalopathy (LATE). In most neurodegenerative diseases there is topographic spreading of misfolded protein pathology between synaptically-connected brain regions with different conformations of the same misfolded protein generally leading to specific patterns of regional and cellular vulnerability. Pathology may also develop in-situ because of cell autonomous factors such as high metabolic demand and genetic risk factors (including possible somatic mutations) that render some neurons more susceptible to protein misfolding[2]. Whatever the mechanism, the clinical features are generally determined by the anatomical distribution of neuropathology, meaning that similar clinical phenotypes may result from several different proteinopathies. Due to this phenotypic overlap, accurate diagnosis of many neurodegenerative diseases in life is challenging and neuropathology remains the gold standard for diagnosis. Consequently, systematic brain banking and clinicopathological correlation have been the cornerstone of clinical diagnostic criteria for all the major neurodegenerative diseases.

A practical guide to the neuropathological diagnosis of neurodegenerative diseases

After weighing the brain, the process begins with macroscopic examination (video 1), assessing the dura/leptomeninges and cerebral vasculature noting any surface lesions or gyral patterns of atrophy before the hemispheres are separated along the midline. In a brain bank setting, one hemisphere is typically fixed in formalin before sectioning and the other is dissected fresh and flash frozen. The formalin-fixed hemisphere is dissected by first removing the brainstem and cerebellum. The hemisphere is sliced coronally, the cerebellum is cut in the sagittal plane and the brainstem is sectioned transversely while looking for any focal or diffuse pathology, including any regional atrophy and depigmentation of the substantia nigra and locus coeruleus. Approximately 20 brain regions are routinely sampled for histologic examination with additional regions included if indicated by the clinical history or macroscopic examination.

Formalin-fixed paraffin embedded tissue sections are prepared from each region for histological examination. Haematoxylin and Eosin (H&E) is used for visualising the tissue cytoarchitecture, and distinguishing neuronal loss due to ischaemia or degeneration. It can also detect certain relevant proteinaceous structures such as extracellular amyloid plaques and intraneuronal Lewy bodies, although these are now better identified with specific antibodies by using immunohistochemistry (IHC). Proteins routinely tested by IHC in neurodegenerative cases include:

1. Aβ peptide

2. Tau

3. α-synuclein

4. TDP43

Other commonly used antibodies target ubiquitin and p62 proteins, which accumulate in diverse pathological inclusions and can be a clue to certain degenerative processes. Depending on the clinical scenario and initial histological findings, IHC for other proteins such as prion (PrP^{SC}) or fused in sarcoma (FUS) are performed. Pathologic lesions detected on H&E and IHC are classified based on their location (extracellular or intracellular), the cell types affected (neurons, astrocytes, oligodendrocytes), their

morphology and regional distribution before the final neuropathological diagnosis is made in the context of the clinical history (figure 1).

ESTABLISHED CLINICOPATHOLOGICAL DISEASES

1. Alzheimer's disease

AD is the most common neurodegenerative disease. It typically presents as an amnestic syndrome but less common phenotypes include posterior cortical atrophy, behavioural and dysexecutive syndromes, logopenic variant primary progressive aphasia (PPA) and corticobasal syndrome (CBS)[3]. Although most AD cases are sporadic, early onset (<65 years) AD comprises 2-10% of all cases, of whom 5-10% have an identifiable autosomal dominant mutation in the presenilin (*PSEN1* and *PSEN2*) or amyloid precursor protein (*APP*) genes or *APP* duplication[4]. The observation of high amounts of Aβ in the parenchyma and vasculature of patients who died from iatrogenic CJD several decades after receiving cadaver-derived human growth hormone treatment[5], has led to widespread recognition that iatrogenic transmission of Aβ neuropathology is also possible during surgical procedures involving contaminated dura mater grafts used in a wide range of medical interventions, or involving contaminated neurosurgical instruments.

Macroscopic features

In typical amnestic cases, cortical atrophy tends to be more pronounced in multimodal association cortices and medial temporal structures, particularly the amygdala and hippocampus, with relative sparing of the primary motor and somatosensory cortices[6]. Loss of neuromelanin pigmentation in the locus coeruleus is also frequently encountered.

Histologic features

AD is a mixed proteinopathy because misfolded Aβ and tau pathology are found together in neuritic plaques (figure 2). Initially, extracellular Aβ plaques are diffuse but as they mature, they develop a

dense central core. In line with the widely accepted amyloid-cascade hypothesis, dense core plaques initiate tau aggregation within surrounding dystrophic neurites culminating in the appearance of neuritic plaques. This hypothesis is based on the finding that mutations in the *APP*, *PSEN1* or *PSEN2* genes, which result in primary abnormalities of Aβ metabolism, can cause young-onset AD whereas mutations in the microtubule associated protein tau (*MAPT*) gene responsible for primary tauopathies do not cause AD[7]. Paramount for AD histological diagnosis is phosphorylated tau aggregation in neuronal bodies leading to the appearance of pre-tangles, which mature into neurofibrillary tangles (NFTs), and following cell death remain as ghost tangles. Although Aβ may be responsible for the initiation of tau misfolding in AD, the degree of cortical atrophy and clinical features correlate with the density of tau pathology rather than that of Aβ[8]. For most cases of AD, the topographical spread of neuropathology follows the model proposed by Braak and Braak, beginning in the medial temporal structures before extending into neocortical structures[9] with the exceptions of rare limbic-predominant and hippocampal-sparing AD. Currently, the neuropathological diagnosis of AD is based on the National Institute on Aging-Alzheimer's Association (NIA) guidelines using semi-quantitative assessments of regional Aβ pathology (Thal phase), regional neurofibrillary tangle presence (Braak and Braak stage) and cortical neuritic plaque (CERAD score) density[10] to indicate the likelihood of dementia being due to AD neuropathological change.

2. Synucleinopathies

The synucleinopathies can be divided into two pathological entities: Lewy body disease (LBD) and multiple system atrophy (MSA). LBD comprises Parkinson's Disease (PD) and Dementia with Lewy bodies (DLB), which are akinetic-rigid syndromes classically separated by the timing of dementia onset, which may occur any time >1 year after the onset of motor symptoms in PD but occurs before or contemporaneously with motor symptoms in DLB. Despite this clinical distinction, PD and DLB likely represent the same clinicopathological continuum. MSA typically presents with autonomic failure and either cerebellar ataxia (MSA-C) or parkinsonism (MSA-P). While there are numerous monogenic

causes (e.g. *SNCA*, *PRKN*, *PINK-1*, *DJ-1*, *VPS35* mutations) and susceptibility genes (*LRRK2*, *GBA*) associated with Parkinson's Disease, MSA is largely a sporadic disease, although combined α-synucleinopathy comprising neuropathological features of both PD and MSA is characteristic of G51D mutations in the *SNCA* gene[11].

Macroscopic examination

The macroscopic findings in PD and DLB are pallor and atrophy of the substantia nigra (SN) and locus coeruleus with variably prominent frontal and medial temporal lobe atrophy[12]. In MSA, atrophy usually extends beyond the SN in olivopontocerebellar- (OPCA) or striatonigral- (SND) predominant patterns that broadly correspond to the cerebellar (MSA-C) and parkinsonian (MSA-P) clinical subtypes respectively. In rare cases, referred to as minimal change MSA, atrophy is limited to the SN despite more widespread misfolded α-synuclein pathology[13].

Histologic examination

PD and DLB are characterised by the accumulation of α-synuclein fibrils in the cytoplasm and processes of neurons forming Lewy bodies and Lewy neurites respectively (figure 3). Lewy pathology tends to evolve in a stereotyped pattern described by Braak and colleagues with characteristic caudal-rostral propagation from the brainstem, through limbic regions to the neocortex. More recently, amygdala-predominant and olfactory-restricted patterns of Lewy pathology are recognised[14]. PD and DLB cannot be distinguished on an individual level at autopsy, consistent with the recent finding that the electron cryo-microscopy (cryo-EM) structures of α-synuclein filaments from patients with these conditions are identical[15]. In MSA, α-synuclein aggregation characteristically occurs in both neurons and oligodendrocytes forming glial cytoplasmic inclusion (GCI) in the latter (figure 3). Inclusions are also seen less frequently within the nuclei of glial cells and neurons. Lewy pathology may be present in 10-20% of MSA cases but the structure of α-synuclein filaments in MSA is distinct from that seen in PD/DLB[16].

Case 1

A 58-year-old man presented with a one-year history of difficulty getting in and out of his bed and car, incomplete bladder emptying, poor stream, double voiding, and hesitancy. Within a few months he developed a shuffling gait and a tendency to drag his left foot leading to several falls. This was followed by tremor of his left hand, hypophonia, constipation, erectile dysfunction, orthostatic light-headedness, and emotional lability. Examination revealed normal eye movements, a mild resting tremor of the left hand with cogwheel rigidity and bradykinesia. He walked with a stooped posture, reduced arm swing and a shuffling gait. There was some improvement of his tremor with levodopa. Repeat examination 18 months into the illness revealed mild dysarthria, ideomotor apraxia and impaired 2-point discrimination and astereognosis affecting his left hand and apraxia of his left leg. There was further rapid progression over the next 6 months with increasing falls, ideomotor apraxia involving the right hand, dysphagia, symptoms suggestive of REM sleep behaviour disorder, left-hand utilisation behaviour and alien limb, episodes of stridor and recurrent chest infections. He died age 71 following a disease duration of 3.5 years and with a final clinical diagnosis of CBS. Neuropathological examination revealed widespread glial cytoplasmic inclusion and neuronal α-synuclein pathology consistent with a diagnosis of MSA (figure 4), which very rarely presents as a CBS. Despite the prominent cortical signs, there was no apparent macroscopic atrophy in any grey or white matter regions, in keeping with minimal change MSA, which is a rare subtype that may be associated with more rapid clinical progression[13].

3. Tauopathies

The primary tauopathies comprise Pick's disease (PiD), Progressive Supranuclear Palsy (PSP), Corticobasal Degeneration (CBD), Globular Glial Tauopathy (GGT) and Chronic Traumatic Encephalopathy (CTE). Argyrophilic Grain Disease (AGD), Primary Age-Related Tauopathy (PART) and ARTAG are common pathologies seen in ageing brains and are described below. PSP classically

presents with Richardson's syndrome, but other clinical phenotypes include parkinsonian and cortical variants. CBD can manifest as CBS, PPA, and even Richardson's syndrome, while GGT presents with varying degrees of frontotemporal dementia (FTD) and upper motor neuron features with or without parkinsonism. PiD typically presents as behavioural variant FTD (bvFTD) but rarely manifests as PPA or CBS[17]. CTE occurs in people with a history of repetitive head impacts and is characterised by progressive episodic memory and/or executive dysfunction, with or without neurobehavioral dysregulation[18,19]. Mutations in the *MAPT* gene produce tauopathies that often have histological features that allow them to be differentiated from the sporadic diseases mentioned above.

Macroscopic examination

The typical macroscopic findings in PSP are atrophy and pallor of the substantia nigra, and atrophy of the subthalamic nucleus, cerebellar dentate nucleus, and superior cerebellar peduncle. Variable atrophy of the neocortex, with characteristic emphasis in the frontal lobe may also be present. The pattern of regional atrophy is more variable in CBD but asymmetric focal cortical atrophy and depigmentation of the substantia nigra are the most common findings. 'Knife edge' atrophy localised to the frontal and temporal lobes with an anteroposterior gradient is classically associated with PiD. CTE is not associated with a distinct macroscopic atrophy pattern but a cavum septum pellucidum is frequently present and there may also be medial temporal lobe atrophy.

Histologic examination

Despite the clinical overlap between these conditions, they can be distinguished by differences in the morphology (figure 5) and regional distribution of tau pathology. PiD is characterised by neuronal cytoplasmic inclusions known as Pick bodies with fewer globular inclusions in glial cells, whereas PSP, CBD and GGT are defined by the morphology of their astrocytic tau inclusions. In PSP, tau accumulates within astrocytic cell bodies resulting in lesions known as tufted astrocytes, whereas tau accumulates in distal astrocytic processes in CBD leading to astrocytic plaques. GGT gets its name from the

characteristic globular tau inclusions seen in astrocytes and oligodendrocytes. The pathognomonic feature of CTE is the presence of neuronal and astrocytic tau pathology distributed around small blood vessels at the depths of cortical sulci, where the greatest mechanical deformation is predicted to occur during head impacts[20]. Neuronal tau pathology also occurs in PSP, CBD, GGT and CTE in the form of neurofibrillary tangles and pre-tangles. Tauopathies can also be classified by the relative abundance of 3- and 4-repeat tau isoforms; PSP, CBD, and GGT have a strong predominance of 4-repeat tau whereas PiD neuronal inclusions are mainly composed of 3-repeat tau. Mixed tauopathies include CTE and AD, which have both 3- and 4-repeat tau pathology[21]. A classification scheme based on the cryo-EM structure of tau filaments from the different tauopathies has also been proposed[22].

Case 2

An 84-year-old man presented with confusion and a 4–5-month history of reduced mobility[23]. He had symmetrical rigidity in the arms more than the legs, difficulty performing finger and foot tapping tasks, and his gait was shuffling and unsteady. It was thought that his cognitive symptoms were exacerbated by prochlorperazine, which was commenced 2 months earlier for vertigo. He was diagnosed with DLB and rivastigmine was started. He subsequently developed low mood with suicidal ideation, anxiety, headaches, pain and tingling in both feet and recurrent falls. Fluctuating cognition, disturbed sleep and physically aggressive behaviour was also noted. He died from his illness age 86. At autopsy, there was no Lewy pathology but rather there was tau pathology consistent with CTE (figure 6). The patient was a former professional association football player and was likely exposed to repetitive head impacts. Although neuropathological examination is required to confirm the diagnosis, this case highlights the importance of seeking a history of repetitive head impacts. Identifying such cases is crucial for determining genetic and environmental factors that may predispose certain individuals to this potentially avoidable condition.

4. TDP43 proteinopathies

Frontotemporal lobar degeneration (FTLD) is characterised by alterations in behaviour/personality and language dysfunction, with relative preservation of episodic memory. Clinically, it can be subdivided into bvFTD and PPA. TDP43 pathology underpins approximately 45% of FTLD cases, with various tauopathies being responsible for most of the remaining cases[24]. Motor neuron diseases including amyotrophic lateral sclerosis (ALS), primary lateral sclerosis (PLS) and progressive muscular atrophy (PMS), comprise the other major group of conditions associated with TDP43 pathology. There is considerable clinical overlap between FTLD-TDP43 and ALS corresponding to the distribution of TDP43 pathology[25]. Specifically, about 15% of individuals with FTLD develop MND, while executive dysfunction and non-executive cognitive impairment may occur in 20-25% and 5-10% of ALS cases respectively[26].

Macroscopic examination

The macroscopic findings in the MND spectrum disorders include atrophy of the motor cortex, spinal cord, and anterior spinal nerve roots, while macroscopic examination of FTLD shows atrophy of the frontal and temporal regions, often accompanied by atrophy and pallor of the substantia nigra.

Histologic examination

On microscopic examination there is loss of Betz cells in the motor cortex and neuronal loss and gliosis in the 12th cranial nerve nucleus, anterior horns, and gliosis in the corticospinal tracts in the brain and lateral columns of the spinal cord.

There is diffuse nuclear immunopositivity for TDP43, an RNA/DNA-binding protein under physiological conditions. In pathogenic states, TDP43 becomes hyperphosphorylated and ubiquitinated, and is mis-localised from the nucleus to the cytoplasm where it forms inclusions (figure 7)[24]. Neuronal cytoplasmic inclusions dominate in both FTLD and MND cases, but rarer neuronal intranuclear and glial inclusions may also be present[27]. FTLD-TDP43 is subdivided into 5 subtypes (type A-E) based on

the patterns of cytoplasmic or intranuclear pathology and cortical distribution[28,29]. Type A, which typically presents as primary non-fluent aphasia (PNFA) or bvFTD, is characterised by numerous short dystrophic neurites, compact or crescentic neuronal cytoplasmic inclusions (NCIs) and neuronal intranuclear inclusions (NIIs) that concentrate in layers II and III of the neocortex. Type B is the most frequent subtype seen in FTLD cases with MND and features compact NCIs that are diffusely distributed throughout all cortical layers, with relatively few dystrophic neurites. Type C tends to cause semantic dementia and is characterised by long, thick dystrophic neurites seen in superficial cortical layers and well-circumscribed NCIs in the hippocampus, amygdala, and basal ganglia. Numerous NIIs are seen in cortical and subcortical regions in Type D, which is associated with the syndrome of inclusion body myositis, Paget's disease of bone, and FTLD. Finally, type E causes rapidly progressive FTD or MND with granulofilamentous neuronal cytoplasmic inclusions seen against a background of grain-like TDP43 deposits in neocortical and subcortical regions. Mutations in the progranulin (*GRN*) and *C9orf72* genes can produce both type A and type B pathology, while p62 and valosin-containing protein (*VCP*) gene mutations can lead to type D pathology. No genetic substrate for type C and E pathology has been identified to date.

Case

A 64-year-old man presented with episodic memory problems, anomia, and subtle semantic language deficits. His mother was diagnosed with AD aged 72 but there was no other known family history of neurological disease. Neuropsychometric testing identified poor verbal recognition memory, impaired naming, reduced semantic fluency and impaired executive function. MRI of his brain demonstrated generalised volume loss that was more pronounced in both hippocampi. He was diagnosed with AD and enrolled in an Aβ monoclonal antibody trial. Within 2 years, he developed behavioural change with prominent aggression. He died age 73. Neuropathological examination revealed widespread TDP43 pathology in keeping with Type A TDP43 proteinopathy. In addition, there were diffuse TDP43 "star-like" perinuclear inclusions and widespread perinuclear p62 immunoreactive inclusions, which

outnumbered TDP43 inclusions in the dentate gyrus and cerebellar granule cells (figure 8). These histological features are highly suggestive of a *C9orf72* hexanucleotide repeat expansion, which was subsequently confirmed by genetic sequencing. FTLD is the most heritable neurodegenerative disease with approximately 18% having a monogenic cause due to mutations in *MAPT* (tauopathies), *GRN*, *VCP* or *C9orf72* repeat expansions (TDP43 proteinopathies)[30]. This case demonstrates how the recognition of a distinctive pattern of pathology led to a post-mortem genetic diagnosis that was unsuspected in life but had implications for the patient's family.

5. Prion diseases

Approximately 85-90% of prion diseases are sporadic, with the remaining familial cases caused by autosomal dominant mutation in the *PRNP* gene that encodes the PrP^C protein[31]. <1% cases are acquired through iatrogenic transmission from PrP^{Sc}-contaminated surgical instruments, cadaveric transplant of dura mater tissue, medical treatment with cadaver derived growth hormone, or dietary exposure to pathological bovine PrP through consumption of contaminated meat (bovine spongiform encephalopathy transmission to humans causing variant CJD) or ritual cannibalism (kuru)[31].

Sporadic Creutzfeldt-Jakob Disease (CJD), the commonest clinical manifestation of prion disease, typically presents as a rapidly progressive dementia variably associated with ataxia, myoclonus, pyramidal and extrapyramidal features. In most cases, death occurs within one year. Several variants are recognised including the Heidenheim visual variant. Genetic cases due to mutations in the *PRNP* gene may present with ataxia (Gerstman-Straussler-Scheinker syndrome) or sleep impairment and distal pain with or without autonomic symptoms (familial fatal insomnia).

Macroscopic examination

There may be some enlargement of the ventricles along with variable atrophy of the cortex and thalamus and in genetic prion disease with predominant ataxia, the cerebellar cortex shows marked

atrophy. However, compared to other more protracted neurodegenerative diseases, the degree of cerebral atrophy is typically mild.

Histologic examination

The hallmark of CJD is spongiform neuropil vacuolation across the grey matter regions. Definitive diagnosis requires demonstration of the abnormal prion protein (referred to as PrP^{Sc}) in the brain or CSF. Depending on the codon 129 variant in *PRNP* and abnormal prion protein conformation it may be distributed in a perineuronal or diffuse synaptic pattern, or as dense-core plaques in grey matter. vCJD may be distinguished by unique involvement of the lymphoreticular system and presence of distinct misfolded prion protein aggregates in the brain.

6. Incidental and concomitant proteinopathies

Except for prion protein, variable amounts of the other above-described misfolded proteins/peptide can be observed in ageing brains and in addition to the primary pathology, most neurodegenerative cases will have varying amounts of one or more other misfolded protein pathologies of uncertain clinical relevance[32]. The prevalence of co-pathology increases with increasing age and may also be associated with *APOE* ε4 genotype[32].

Incidental Lewy body disease:

Community-based population studies indicate that the prevalence of incidental Lewy body disease (iLBD) ranges from 15% to 40% in those aged more than 60 and 85 years old respectively[33,34]. In two thirds of these cases the pattern of Lewy pathology follows the typical caudal-rostral pattern while another third of case have an amygdala-predominant pattern, which is influenced by the *APOE* ε4 genotype. iLBD is associated with reduction in dopaminergic nigral neurons and striatal dopamine levels suggesting that it may represent premotor PD[33].

Primary age-related tauopathy (PART):

PART refers to NFT pathology that is histologically and structurally indistinguishable from that seen in AD but in the absence of any significant amyloid-β pathology[35]. PART is largely restricted to the medial temporal lobes, basal forebrain, brainstem, olfactory bulb and cortex[35]. Compared to AD, PART cases tend to have considerably less tau pathology outside the medial temporal lobe, lower frequency of *APOE* ε4 risk allele, higher frequency of the protective *APOE* ε2 allele and less severe cognitive impairment[36].

Age-related tau astrogliopathy (ARTAG):

ARTAG is a spectrum of astroglial tau pathology mainly seen in individuals over 60 [37]. It may co-exist with other primary tauopathies and is characterised by two distinct patterns of astrocytic tau pathology, referred to as fuzzy/granular and thorn-shaped astrocytes (TSAs), which can develop in grey and white matter regions. TSA pathology in particular can be observed in subpial and perivascular distributions and in isolation, closely mimics astrocytic tau pathology seen in CTE[37]. In one series, TSAs were identified in the frontotemporoparietal subcortical white matter in the majority of pathologically confirmed AD cases with PPA suggesting that ARTAG may be associated with this clinical variant of AD[38].

Argyrophilic grain disease (AGD):

AGD is characterised by the presence of small, grain-like inclusions that are within neuronal dendrites and axons and contain abnormal 4-repeat tau filaments. They are commonly seen in the brains of older individuals, most commonly in the medial temporal lobe, but in advanced cases, also extending to cortical and brainstem regions[39]. AGD is rarely the sole pathology in those with neurological symptoms, rather it tends to co-exist with other neurodegenerative diseases, particularly PSP and CBD. In the context of AD, it has been suggested that co-existing AGD may lower the threshold for AD pathology to manifest with clinical symptoms[40]. As the sole pathology, AGD

may be responsible for mild cognitive impairment, behavioural and psychiatric symptoms in older people and rarely, FTD[39].

Limbic-predominant age-related TDP43 encephalopathy (LATE):

LATE neuropathological change (LATE-NC) refers to TDP43 pathology in older adults, with or without hippocampal sclerosis, which, as the name implies, typically shows emphasis in limbic regions, but in advanced cases can also extend into the frontal cortex. LATE-NC may affect 20-50% of individuals over 80 years. It can cause an amnestic syndrome that mimics AD and is increasingly recognised as a source of considerable morbidity in older adults[41].

DONOR REFERRAL

Patients who would like to find out more about brain donation should be referred to the nearest brain bank. Locations and contact details for brain banks in the UK, as well as useful information for potential donors, can be found online (https://brainbanknetwork.ac.uk/public/donating). Donations from people without symptoms of a neurodegenerative disease should also be encouraged as there is a shortage of brain tissue from healthy control donors. Moreover, increasing diversity in terms of the sex and race of donors will be important to ensure that research is representative of the entire population.

CONCLUSION

Neuropathological examination not only remains the gold standard for reaching a definitive diagnosis in most neurodegenerative diseases, but it can also have important implications for living relatives as well as wider public health implications. New pathological entities requiring further clinicopathological correlation continue to be described and with advances in molecular biology techniques, neuropathological examination of tissue from well-established conditions such as AD and PD remains

as important as ever to facilitate high quality research into the molecular mechanisms of these diseases and for the discovery and validation of tissue biomarkers.

KEY POINTS:

1. Most neurodegenerative diseases are characterised by misfolding of physiological proteins leading to characteristic neuronal and glial inclusions or extracellular deposits that allow these conditions to be distinguished from each other on examination of brain tissues.

2. There is considerable overlap in clinical phenotypes determined by the regional distribution of neuropathology and neuronal loss.

3. Despite advances in neuroimaging and fluid biomarkers, neuropathological examination of the brain remains the gold-standard diagnosis for many neurodegenerative diseases.

4. Subject to appropriate funding, brain banks enable research through careful curation of high-quality CNS and peripheral organ tissues and fluids from longitudinally well-characterised donors with and without neurodegenerative diseases.

5. Neuropathological examination of the brain is not only crucial to advance our understanding of the pathobiological basis of disease and to establish the diagnosis in clinically difficult cases, but may also yield unexpected findings, which may have important implications for living relatives and public health.

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Figure 1 Formation of misfolded protein pathology and overview of pathological classification of neurodegenerative disease.

Figure 2 Morphology of Alzheimer's disease-related tau inclusions and Aβ plaques.

Figure 3 Morphology of α-synuclein inclusions in Parkinson's disease and multiple system atrophy.

Figure 4 Macroscopic and microscopic findings in a case of minimal change multiple system atrophy. Compared to the significant lateral putaminal atrophy seen in a case of MSA-SND (A2, blue arrowhead), there is no macroscopic atrophy of the basal ganglia structures (A1). Similarly, there is no atrophy of the pontine base (A3, vertical line) compared to the severe reduction in the height of this structure in a case of MSA-OPCA (A4, vertical lines). In contrast to atrophy and gliosis of the inferior olivary nucleus in a case of MSA-OPCA (A6, purple arrow), this structure is of normal bulk and well demarcated in this case (A5, blue arrow). The cerebellar white matter is also preserved (A8, green arrow) compared to severe cerebellar white matter atrophy seen in MSA-OPCA (A7, yellow arrow (note good preservation of superior cerebellar peduncle, shown with blue asterisk). Histological examination reveals an α-synucleinopathy with pathological inclusions seen in both glial and neuronal cells. The morphology of glial cytoplasmic inclusions (GCIs) is typical of MSA. Glial cytoplasmic inclusions are seen within pencil fibres of Wilson in the putamen (B1), pontine base white matter (B2), the inferior olivary nucleus (B3) and the cerebellar white matter (B4). Whilst on macroscopic examination, neither striatonigral nor

olivopontocerebellar regions show any apparent atrophy, on histological examination, the lateral part of the posterior putamen shows mild gliosis and neuronal depletion, although the atrophy of the substantia nigra is minimal (not shown). Therefore, the pathology corresponds best to minimal change MSA with histological evidence of striatal (posterior putaminal) atrophy. Moderately severe hyaline arteriolosclerosis was also seen in occasional blood vessels in the cerebral white matter and there was focal cerebral amyloid angiopathy in the occipital lobe involving the occipital cortex and overlying leptomeninges (not shown). CC: corpus callosum; Cau: caudate nucleus; IC: internal capsule; AC: anterior commissure; Put: putamen; GP: globus pallidus. Scale bar: B1, 75µm; B2 and B4, 300µm; B3, 100µm.

Figure 5 Morphology of cellular inclusions in various tauopathies.

Figure 6 Macroscopic and microscopic findings in a case of chronic traumatic encephalopathy. There is mild global cerebral volume loss with enlargement of the lateral ventricle (A1), marked atrophy of the hippocampus (B7), and severe pallor and neuronal loss in the substantia nigra (B10 and B11). Immunohistological examination revealed widespread, irregularly distributed tau pathology in the form of subpial, periventricular and perivascular thorn-shaped astrocytic tau pathology, dense limbic neuronal and glial tau pathology, and patchy cortical neuropil thread, pre-tangle, and tangle pathology particularly in the superficial cortical laminae as shown in the frontal cortex (B1, B4 and B6). There are frequent thorn-shaped astrocytes arranged in an irregular and patchy manner in the cortex, including around cortical blood vessels (B3, red asterisk), with some emphasis at the depths of cortical sulci (B1, blue arrowheads, and B2). Subpial thorn-shaped astrocytes are seen in the neocortex (B5), midbrain (B13) and pons (not shown). Thorn-shaped astrocytes are also present in the subependymal white matter adjacent to the lateral and fourth ventricles (not shown). Numerous pre-tangles and occasional tangles are seen in the dentate gyrus (B9) and pre-tangles and tangles with proximal dendritic swellings are seen in the CA4 region (B8). Occasional tangle, pre-tangle and thread pathology can also

be seen in the substantia nigra (B12). Occasional TDP43 immunoreactive neuronal cytoplasmic inclusions were present in the dentate gyrus (not shown). There was also widespread amyloid-β pathology corresponding to Thal phase 5 (not shown). SFG: superior frontal gyrus; MFG: middle frontal gyrus; IFG: inferior frontal gyrus; CC: corpus callosum; Cau: caudate nucleus; IC: internal capsule; Put: putamen; PHG: parahippocampal gyrus; FG: fusiform gyrus; CA1-4: cornu Ammonis 1-4; CP: cerebral peduncle; MGN: medial geniculate nucleus; SN: substantia nigra; RN: red nucleus; SC: superior colliculus. Scale bar: B1, 5mm; B2, 940μm; B3, 65μm; B4, 750μm; B5, 470μm; B6, 570μm; B7, 6mm; B8 and B9, 70μm; B10, 6mm; B11, 300μm; B12, 70μm; B13, 210μm.

Figure 7 Morphology of cellular inclusions in TDP43 proteinopathies.

Figure 8 Macroscopic and microscopic findings in a case of FTLD-TDP type A due to *C9orf72* hexanucleotide repeat expansion. There is severe atrophy of the hippocampus, with less prominent atrophy of the frontal lobe (A1). There was also prominent atrophy of the anterior temporal lobe with reduction in size of the amygdala, with less prominent atrophy of the parietal lobes, and reduction in bulk of the caudate nucleus, globus pallidus (not shown) and thalamus (A1, purple arrow). Frequent p62 immunoreactive perinuclear inclusions (green arrowheads) are seen in the superficial layers of frontal cortex (B1-2), dentate gyrus of the hippocampus (B4-5) and in cerebellar granule cells (B7-8). Frequent short TDP43 immunoreactive neurites (B3, blue arrowhead) were seen predominantly in the superficial cortex corresponding to layer 2. The cytoplasmic neuronal inclusions (B3 and B6, red arrowheads) although seen in a pan-cortical distribution, showed emphasis in superficial cortical layers, in keeping with type A TDP43 proteinopathy. TDP43 and p62 pathology was also present in the parietal, lateral temporal, and insular cortices, and in the putamen and caudate nucleus (not shown). Importantly, the perinuclear, granular dot-like (also called "star"-like) p62 immunoreactive inclusions (B2, B5 and B8, green arrowheads) were more frequent than TDP43 positive inclusions in the dentate

gyrus (B6, red arrowhead) and in the cerebellar granule cells where there are no pathological TDP43 immunoreactive aggregates (B9). These histological features are highly suggestive of primary TDP43 proteinopathy due to a pathological *C9orf72* hexanucleotide repeat expansion, which was confirmed by genetic testing. There was also intermediate level Alzheimer's disease pathological change, a chronic cortical microinfarct (anterior medial temporal lobe), patchy cerebral cortical and leptomeningeal amyloid angiopathy, and moderate hyaline arteriolosclerosis with mild atheroma (not shown). Scale bar: B1, 5mm; B2, B3, B5, B6, B8, B9, 25μm; B4, 3mm; B7, 4mm.