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Neurodegenerative diseases

PREVALENCE OF ALZHEIMER-TYPE PATHOLOGY IN NON-ALZHEIMER NEURODEGENERATIVE DISORDERS AND ITS IMPLICATION FOR THE DEVELOPMENT OF BIOMARKERS: A BRAIN-BANK-BASED POST-MORTEM STUDY

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1. Summary

The main objective of this project was to identify the frequency and severity of concomitant Alzheimer's disease (AD)-pathology in non-AD neurodegenerative diseases. This is important as CSF biomarkers in AD are based on the detection of proteins that abnormally accumulate in the brain such as beta-amyloid and phospho-tau. These markers have been also investigated in other neurodegenerative diseases, to evaluate their usefulness to infer the underlying neuropathology and potential contribution to cognitive decline. However, the frequent coexistence of AD pathology in non-AD neurodegenerative diseases may influence their proper interpretation in the clinical work-up of cognitive decline. A brain-bank-based study offered a unique frame to better characterize diseases from a neuropathological point of view, increasing their added value for researchers to whom samples will be provided in the future.

Therefore, we analyzed a well characterized, brain-bank-based post-mortem series of non-AD neurodegenerative diseases by histology and immunohistochemistry for different abnormally accumulated proteins in the brain (beta-amyloid, phospho-tau, 3R-4R-tau isoforms, alpha-synuclein, TDP43, FUS) and performed a semiquantitative assessment of the density of protein aggregates in multiple brain regions to identify the frequency, regional distribution, and overall density of concomitant AD-pathology. We also performed a correlation analysis of local and total AD pathology load with clinical data and ApoE genotype in a subgroup of pathologies. Another aim was to analyze a prospective brain donor series that had been tested for AD biomarkers in CSF during life. This analysis was done partially, as the number of brain donations of these patients was lower than expected.

2. Results

We have analyzed the presence and distribution of AD pathology in the following retrospective series of our brain bank:

***1. Alpha-synucleinopathies**:*

I. Parkinson disease with and without dementia
II. Genetic Lewy body diseases (LRRK2 mutations)

***2. Tauopathies**:*

I. 4-repeat tauopathies: Progressive supranuclear palsy
II. Corticobasal degeneration
III. Genetic forms with MAPT mutations (P301L)
IV. Other tauopathies: IgLON5 tauopathy

***3. TDP43 proteinopathies**:*

I. FTLD: Fronto-temporal lobar degenerations with TDP43 protein aggregates
II. ALS
III. Genetic FTLD/ALS with C9orf72 expansion

4. FUSopathies:

I. ALS with FUS+ protein aggregates
II. FTLD with FUS+ protein aggregates

5. Prion diseases:

I. Creutzfeldt-Jakob disease, sporadic and genetic
II. Variably protease sensitive prionopathy

Details of selected studies:

1. Alpha-synucleinopathies

I. <u>Parkinson disease with and without dementia</u>

a) We performed a semiquantitative detailed mapping of α-synuclein, tau, β-amyloid (Aβ), TDP-43, and AGD pathologies in 17 areas in 63 LBD cases (44 with Parkinson disease [PD], 28 with dementia, and 19 with dementia with Lewy bodies). APOE and MAPT genetic variants were also investigated. A majority of LBD cases had 2 or 3 concomitant findings, particularly Alzheimer disease-related pathology. Pathological stages of tau, β-amyloid and α-synuclein pathologies were increased in cases with dementia. Aβ score was the best correlate of the time to dementia in PD. In addition, β-amyloid deposition correlated with α-synuclein load in all groups. MAPT H1 haplotype did not influence any assessed pathology in PD. These results highlight the common concurrence of pathologies in patients with LBD that may have an impact on the clinical expression of the diseases.

b) In a second study we also observed that a-synuclein midbrain scores rose from controls to AD and then LBD irrespective of dementia. Aβ and tau were more prominent in the tectum/tegmentum, increasing from controls to LBD (mostly in dementia cases in the case of Aβ), and then peaking in AD. By contrast, cerebellar Aβ scores were marginal across the LBD-spectrum, as opposed to AD, only showing a trend towards greater involvement in LBD cases with dementia.

2. Tauopathies

I. 4-repeat tauopathies: Progressive supranuclear palsy

a) We participated in an international multisite study assessing the clinical features and investigations that predict or exclude PSP pathology during life, aiming at an optimization of the clinical diagnostic criteria for PSP.

b) We assessed PSP pathology scores (neuronal/glial; widespread/subcortical), concomitant pathologies and genetic determinants (APOE genotype and MAPT haplotype) in 67 PSP cases from our brain bank. Most cases (94%) featured copathology, with AD changes being the most frequent (73.1%), followed by argyrophilic grains (23.9%), vascular pathology (22.4%), PART (16.4%), Lewy body pathology (13.4%) and TDP43 pathology (7.5%). PSP total and dentate nucleus scores were significantly higher in patients with cognitive impairment. Prevalence of CAA was higher in APOE ε4 carriers. Widespread cases had shorter disease duration and higher caudate scores than predominantly subcortical cases. Predominantly glial cases were older, had shorter disease and higher frontal tau scores, whereas predominantly neuronal cases had a greater proportion of PART. (Manuscript in preparation).

II. Genetic forms with MAPT mutations

We studied 13 patients carrying the P301L mutation in the MAPT gene. Neuropathology in 9 cases showed an extensive neuronal and glial 4-repeat (4R) tauopathy with mini-Pick-like bodies in the dentate gyrus as the characteristic underlying pathology in all cases. In 1 subject, additional 4R globular glial inclusions were observed. AD-copathology was overall very low. All the mutation carriers showed the same haplotype for the SNPs analyzed, suggesting a common ancestor.

III. Other tauopathies: IgLON5 tauopathy

We defined neuropathological criteria of a novel tauopathy associated with autoantibodies, named IgLON5 tauopathy. We provide diagnostic levels of certainty based on the presence of associated clinical and immunological data (definite, probable and possible). The brains of 6 patients showed a neurodegenerative disease, absence of inflammatory infiltrates and neuronal accumulation of both three-repeat (3R) and four-repeat (4R) tau isoforms, preferentially involving the hypothalamus, and more severely the tegmental nuclei of the brainstem. Concomitant AD pathology was also present in a small fraction of cases. These criteria should help to identify undiagnosed cases among archival tissue, and will assist future clinical/pathological studies of this novel disorder.

3. TDP43 proteinopathies

I. FTLD: Fronto-temporal lobar degenerations with TDP43 protein aggregates

A) We assessed the FTD Consortium revised criteria of behavioural variant frontotemporal dementia (bvFTD) in a pathological cohort and to determine their predictive values in a clinical context suggestive of bvFTD. In addition, the study aimed to assess the influence of the age at onset and underlying pathology in the clinicopathological correlations. The FTDC criteria reached a sensitivity of 93% for possible and 80% for probable bvFTD. Early-onset cases displayed significantly more disinhibition, loss of empathy and compulsive behaviour with respect to late-onset bvFTD, leading to a slightly higher sensitivity of the diagnostic criteria (97% vs. 91%). There were no differences in the diagnostic performance between tau-positive and tau-negative cases. In subjects clinically diagnosed as bvFTD, a possible bvFTD diagnosis reached a positive predictive value for FTLD pathology of 90%, irrespective of underlying proteinopathy. False-positive clinical diagnoses were mainly Alzheimer's disease. These cases were significantly older, had less family history of dementia and had a predominantly apathetic clinical picture. The revised bvFTD criteria present good sensitivity and positive predictive value in both early- and late-onset cases and regardless of the underlying FTLD pathology.

B) We contributed to a multisite study lead by Prof. D. Bergeron concerning the prevalence of Aß pathology in distinct variants of primary progressive aphasia and the effects of ApoE4 allele. The study included 1,251 patients diagnosed with PPA variants (logopenic, nonfluent, semantic and mixed/unclassifiable) from 36 centers. The study

showed that amyloid-β positivity was more prevalent in lvPPA (86%) than in nfvPPA (20%) or svPPA (16%; $p < 0.001$). Prevalence of amyloid-β positivity increased with age in nfvPPA (from 10% at age 50 years to 27% at age 80 years, $p < 0.01$) and svPPA (from 6% at age 50 years to 32% at age 80 years, $p < 0.001$), but not in lvPPA ($p = 0.94$). Across PPA variants, ApoE ε4 carriers were more often amyloid-β positive (58.0%) than non-carriers (35.0%, $p < 0.001$). Autopsy data revealed Alzheimer disease pathology as the most common pathologic diagnosis in lvPPA (76%), frontotemporal lobar degeneration–TDP-43 in svPPA (80%), and frontotemporal lobar degeneration–TDP-43/tau in nfvPPA (64%). This study shows that the current PPA classification system helps to predict underlying pathology across different cohorts and clinical settings, and suggests that age and ApoE genotype should be considered when interpreting amyloid-β biomarkers in PPA patients.

II. ALS

We have analyzed 86 ALS cases for the extent of concomitant pathologies and their contribution to cognitive decline. Mean age at onset of motor neuron symptoms was 63.2 years (range 29 - 86). Cognitive decline was recorded during the disease in 27 subjects (31.4%). Mutations were present in 12 subjects (8 C9orf72, 2 TARDBP, 1 TBK1, 1VCP), and most of them (83%) developed cognitive symptoms during the disease. ApoEe4 carriers had the highest AD pathology scores. LBs were observed in a small fraction of cases, as well as AgD and PART. Patient with cognitive decline had higher Brettschneider stages of ALS. In this preliminary analysis we observed that cognitive decline is a frequent finding in ALS. In our series, the neuropathological substrate seems to be mixed and it is not only contributed by the extramotor extent of TDP43 and the presence of hippocampal sclerosis but also by relatively early PART and argyrophilic grain pathology, and Lewy-body and AD-pathology in older age groups. (Manuscript in preparation)

III. Genetic FTLD/ALS with C9orf72 expansion

Screening of the cerebellar cortex of our adult brain bank cohort (n=1500) with ubiquitin/p62 immunohistochemistry to identify potential C9orf72 expansion mutation carriers in addition to those donors already diagnosed during life. We identified a total of 21 cases with the expansion mutation. In these cases, we analysed the presence of TDP43, alpha-internexin, beta-amyloid, tau and alpha-synuclein pathology in several brain areas. We identified the presence of Lewy body type alpha-synuclein pathology in

three cases that likely influenced the clinical phenotype. Moreover, a relatively high percentage also had neuropathological features of primary age-related tauopathy, besides the TDP43 pathology.

5. Prion diseases

I. Creutzfeldt-Jakob disease, sporadic and genetic

We described clinical/-pathological features, including Alzheimer's copathology, and diagnostic accuracy in our postmortem series of 160 rapidly progressive dementia (RPD) cases from 2001-2011. Prion diseases were the most frequent neuropathological diagnosis (67%), followed by non-prion neurodegenerative pathologies (17%), mostly AD and dementia with Lewy bodies, and non-neurodegenerative diseases (16%). Four patients with potentially treatable disorders were diagnosed, while still alive, as having Creutzfeldt-Jakob disease. Concomitant pathologies were detected in 117 (73%). Among all RPD cases, 51 presented moderate or frequent mature β-amyloid plaques (neuritic plaques), which are considered to be associated with positive amyloid biomarkers in vivo. The presence of concomitant pathologies, mainly Alzheimer's disease (AD), may act as a confounding variable in the diagnostic process.

3. Relevance and possible clinical applicability of the final results

We believe that our results shed some light on the effects of brain proteins, their frequency and their relation with clinical diagnostic tools and diagnostic criteria, which will ultimately influence therapeutic decisions.

We have observed that Alzheimer-type pathology is an important contributor of pathology load in the brain in several neurodegenerative diseases, especially in Lewy body and prion disorders and, in particular, in older age groups. In FTLD, but especially in primary tauopathies, the effect seems to be less, e.g. the PSP pathology burden and distribution appear to be more relevant for demographic or clinical features in PSP than copathology or genetic determinants. In ALS we observed that cognitive decline is a frequent finding and the neuropathological substrate seems to be mixed and is not only contributed by the extramotor extent of TDP43 and the presence of hippocampal sclerosis but also by relatively early PART and argyrophilic grain pathology, and Lewy-body and AD-pathology in older age groups. This is also true for C9orf72 expansion

mutation carriers. The presence of AD pathology likely influences or at least modulates the clinical phenotype in several diseases, making the clinical diagnosis of a primary pathology even more difficult. These findings support the view of the increasing awareness of the concept of brain multimorbidities or multiproteinopathies.

We have confirmed that post-mortem neuropathological studies are a very valuable source of additional information that cannot be obtained in vivo and that help to clarify and expand the clinical diagnosis and the aetiology of dementia, facilitating complementary molecular studies. Early pathologic changes in the brain (morphological and biochemical) are largely unknown for most neurodegenerative diseases. Every effort directed to a better understanding of the meaning of biological processes that lead to neurodegeneration is important. Brain banks offer a unique possibility to better characterize the diseases from a neuropathological point of view, and also genetically by increasing their added value for researchers to whom samples will be provided in the future.

The information obtained from detailed postmortem neuropathological studies contributes to a more appropriate interpretation of currently available biomarkers (e.g. PET amyloid imaging, CSF biomarkers) as it is getting more and more crucial to stratify patients as correctly as possible for the appropriate investment in and optimization of diagnostic resources and treatment strategies. Moreover, it contributes to the improvement in the field of biomarker research and their optimal translation into daily clinical practice. Furthermore, the fact that advancing age and carrying the APOEe4 allele harbour a risk of increased proteinopathies will be of relevance when specific treatments for specific proteins are available, such as anti-tau, ßAmyloid, alpha-synuclein or TDP43, for which there are already some ongoing trials.

4. Publications, communications

We have published 12 articles (and 3 additional without citation of the Fundació la Marató de TV3), 8 of them are positioned in Q1 Pathology Clinical Neurology and/or Neuroscience and have a total impact factor of 68,65. The articles are indexed in the Web of Science and have been presented at several international congresses or

conferences (one was awarded the best poster prize). Our work has already received 96 citations by ISI Web of Science and 144 citations by Google Scholar.

A) Publications with acknowledgements to the Fundació Marató de TV3 (chronological order):

1) Grau-Rivera O, **Gelpi E**, Nos C, Gaig C, Ferrer I, Saiz A, Lladó A, Molinuevo JL, Graus F, Sánchez-Valle R; Neurological Tissue Bank Collaborative Group. ***Clinicopathological Correlations and Concomitant Pathologies in Rapidly Progressive Dementia: A Brain Bank Series. N***eurodegener Dis. 2015;15(6):350-60.

2) Sierra M, **Gelpi E,** Martí MJ, Compta Y. ***Lewy- and Alzheimer-type pathologies in midbrain and cerebellum across the Lewy body disorders spectrum***. Neuropathol Appl Neurobiol. 2016 Jan 26. doi: 10.1111/nan.12308.

3) Balasa M, **Gelpi E,** Martín I, Antonell A, Rey MJ, Grau-Rivera O, Molinuevo JL, Sánchez-Valle R, Lladó A; Catalan collaborative Study Group for FTLD. ***Diagnostic accuracy of behavioral variant frontotemporal dementia consortium criteria (FTDC) in a clinicopathological cohort.*** Neuropathol Appl Neurobiol. 2015 Dec;41(7):882-92.

4) Colom-Cadena M, Grau-Rivera O, Planellas L, Cerquera C, Morenas E, Helgueta S, Muñoz L, Kulisevsky J, Martí MJ, Tolosa E, Clarimon J, Lleó A, **Gelpi E***. **Regional Overlap of Pathologies in Lewy Body Disorders***. J Neuropathol Exp Neurol. 2017 Mar 1;76(3):216-224.

5) Compta Y, Ramos-Campoy O, Grau-Rivera O, Colom-Cadena M, Clarimón J, Martí MJ, **Gelpi E.** ***Conjoint FTLD-FUS of the neuronal intermediate filament inclusion disease type, progressive supranuclear palsy and Alzheimer's pathology presenting as parkinsonism with early falls and late hallucinations, psychosis and dementia*****.** Neuropathol Appl Neurobiol. 2017 Jun;43(4):352-357.

6) **Gelpi E,** Höftberger R, Graus F, Ling H, Holton JL, Dawson T, Popovic M, Pretnar-Oblak J, Högl B, Schmutzhard E, Poewe W, Ricken G, Santamaria J, Dalmau J,

Budka H, Revesz T, Kovacs GG. ***Neuropathological criteria of anti-IgLON5-related tauopathy.*** Acta Neuropathol. 2016 Oct;132(4):531-43.

7) Borrego-Écija S, Morgado J, Palencia-Madrid L, Grau-Rivera O, Reñé R, Hernández I, Almenar C, Balasa M, Antonell A, Molinuevo JL, Lladó A, Martínez de Pancorbo M, **Gelpi E,** Sánchez-Valle R. ***Frontotemporal Dementia Caused by the P301L Mutation in the MAPT Gene: Clinicopathological Features of 13 Cases from the Same Geographical Origin in Barcelona, Spain*.** Dement Geriatr Cogn Disord. 2017;44(3-4):213-221.

8) Borrego-Écija S, Cortés-Vicente E, Cervera-Carles L, Clarimón J, Gámez J, Batlle J, Ricken G, Molina-Porcel L, Aldecoa I, Sánchez-Valle R, Rojas-García R, **Gelpi E**. ***Does ALS-FUS without FUS mutation represent ALS-FET? Report of three cases***. Neuropathol Appl Neurobiol. 2018 Oct 29.

9) Vicente-Pascual M, Rossi M, Gámez J, Lladó A, Valls J, Grau-Rivera O, Ávila Polo R, Llorens F, Zerr I, Ferrer I, Nos C, Parchi P, Sánchez-Valle R, **Gelpí E.** ***Variably protease-sensitive prionopathy presenting within ALS/FTD spectrum.*** Ann Clin Transl Neurol. 2018 Sep 21;5(10):1297-1302. doi: 10.1002/acn3.632. eCollection 2018 Oct.

10) Ramos-Campoy O, Ávila-Polo R, Grau-Rivera O, Antonell A, Clarimón J, Rojas-García R, Charif S, Santiago-Valera V, Hernandez I, Aguilar M, Almenar C, Lopez-Villegas D, Bajo L, Pastor P, Van der Zee J, Lladó A, Sanchez-Valle R, **Gelpi E.** ***Systematic Screening of Ubiquitin/p62 Aggregates in Cerebellar Cortex Expands the Neuropathological Phenotype of the C9orf72 Expansion Mutation.*** J Neuropathol Exp Neurol. 2018 Aug 1;77(8):703-709.

11) Cortés-Vicente E, Turon-Sans J, **Gelpi E**, Clarimón J, Borrego-Écija S, Dols-Icardo O, Illán-Gala I, Lleó A, Illa I, Blesa R, Al-Chalabi A, Rojas-García R. ***Distinct Clinical Features and Outcomes in Motor Neuron Disease Associated with Behavioural Variant Frontotemporal Dementia.*** Dement Geriatr Cogn Disord. 2018 Jun 8;45(3-4):220-231.

12) Respondek G, Kurz C, Arzberger T, Compta Y, Englund E, Ferguson LW, **Gelpi E**, Giese A, Irwin DJ, Meissner WG, Nilsson C, Pantelyat A, Rajput A, van Swieten JC, Troakes C, Josephs KA, Lang AE, Mollenhauer B, Müller U, Whitwell JL, Antonini A, Bhatia KP, Bordelon Y, Corvol JC, Colosimo C, Dodel R, Grossman M, Kassubek J, Krismer F, Levin J, Lorenzl S, Morris H, Nestor P, Oertel WH, Rabinovici GD, Rowe JB, van Eimeren T, Wenning GK, Boxer A, Golbe LI, Litvan I, Stamelou M, Höglinger GU; Movement Disorder Society-Endorsed PSP Study Group. ***Which ante mortem clinical features predict progressive supranuclear palsy pathology?*** Mov Disord. 2017 Jul;32(7):995-1005.

Collaborations within the same research line in which Fundació Marató de TV3 was unfortunately not mentioned:

1) Bergeron D, Gorno-Tempini ML, Rabinovici GD, Santos-Santos MA, Seeley W, Miller BL, Pijnenburg Y, Keulen MA, Groot C, van Berckel BNM, van der Flier WM, Scheltens P, Rohrer JD, Warren JD, Schott JM, Fox NC, Sanchez-Valle R, Grau-Rivera O, **Gelpi E**, Seelaar H, Papma JM, van Swieten JC, Hodges JR, Leyton CE, Piguet O, Rogalsky EJ, Mesulam MM, Koric L, Nora K, Pariente J, Dickerson B, Mackenzie IR, Hsiung GR, Belliard S, Irwin DJ, Wolk DA, Grossman M, Jones M, Harris J, Mann D, Snowden JS, Chrem-Mendez P, Calandri IL, Amengual AA, Miguet-Alfonsi C, Magnin E, Magnani G, Santangelo R, Deramecourt V, Pasquier F, Mattsson N, Nilsson C, Hansson O, Keith J, Masellis M, Black SE, Matías-Guiu JA, Cabrera-Martin MN, Paquet C, Dumuirger J, Teichmann M, Sarazin M, Bottlaender M, Dubois B, Rowe CC, Villemagne VL, Vandenberghe R, Granadillo E, Teng E, Mendez M, Meyer PT, Frings L, Lleó A, Blesa R, Fortea J, Seo SW, Diehl-Schmid J, Grimmer T, Frederiksen KS, Sánchez-Juan P, Chételat G, Jansen W, Bouchard RW, Robert L Jr, Visser PJ, Ossenkoppele R. ***Prevalence of amyloid-β pathology in distinct variants of primary progressive aphasia****.* Ann Neurol. 2018 Sep 26. doi: 10.1002/ana.25333.

2) Vilas D, Sharp M, **Gelpi E**, Genís D, Marder KS, Cortes E, Vonsattel JP, Tolosa E, Alcalay RN. ***Clinical and neuropathological features of progressive supranuclear palsy in Leucine rich repeat kinase (LRRK2) G2019S mutation carriers.*** Mov Disord. 2018 Feb;33(2):335-338.

3) Vilas D, **Gelpi E**, Aldecoa I, Grau O, Rodriguez-Diehl R, Jaumà S, Martí MJ, Tolosa E. ***Lack of central and peripheral nervous system synuclein pathology in R1441G LRRK2-associated Parkinson's disease***. J Neurol Neurosurg Psychiatry. 2018 Jul 27.

Manuscripts in preparation:

- Grau-Rivera O, Compta Y, Tolosa E, Martí MJ, Valldeoriola F, Pagonabarraga J, Calopa M, Bayès A, Hernandez I, Aguilar M, Genis D, Fernandez M, Munoz-Garcia, Cristina, Respondek G, Höglinger G, Antonell A, Gelpi E. ***Concomitant pathologies and genetic determinants in progressive supranuclear palsy****.* In preparation

- Sergi Borrego-Écija, Janina Turón, Teresa Ximelis, Iban Aldecoa, Laura Molina-Porcel, Mónica Povedano, Raquel Sánchez-Valle, Ricardo Rojas-García, Ellen Gelpi. ***Concomitant pathologies and their contribution to cognitive decline in amyotrophic lateral sclerosis***. In preparation