STUDY OF GENETIC FRONTOTEMPORAL DEMENTIA IN PRECLINICAL AND EARLY STAGES OF THE DISEASE: COGNITIVE PERFORMANCE, STRUCTURAL AND FUNCTIONAL NEUROIMAGING AND BIOCHEMICAL MARKERS

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1. Summary of the project and main results

Frontotemporal dementia is the second-commonest neurodegenerative dementia in subjects aged under 65 years. Currently, there are no treatments that have shown a positive effect on the modification of the course of the disease. Finding a cure has the additional difficulty that frontotemporal dementia is not a homogeneous disease but a group of different proteinopathies with similar clinical presentations that are at present very difficult to identify during life. Frontotemporal dementia shows important family aggregation, and it is estimated that up to 40% of cases have family history of disease. In 5-15% of frontotemporal dementia patients a pathogenic mutation can be identified as the cause of the disease. In our population, hexanucleotide expansions in C9ORF72, mutations in the granulin gene (GRN) and mutations in the microtubule associated protein tau (MAPT) are the most frequent causes of genetic frontotemporal dementia. Genetic forms of frontotemporal dementia make it possible, on one hand, to identify the protein causing the disease during life and on the other hand, to study the initial phases of the disease in the brain in mutation carriers, years before the onset of symptoms. Individual forms of genetic frontotemporal dementia are considered rare diseases, meaning that only multicentre studies grouping several referral centres are able to carry out studies in these populations. In this context, the aim of this research project was to characterize the clinical, cognitive, biochemical and neuroimaging markers in mutation carriers compared to non-carrier siblings and to study the changes of these biomarkers over time.

The first result obtained within this project funded by the Fundació La Marató has been the inclusion of our group in the Frontotemporal Genetic Dementia Initiative (Genetic frontotemporal dementia Initiative, GENFI, http://genfi.org.uk/index.html) in its 2nd phase. GENFI is a multicentre project that currently groups 26 European and Canadian centres, led by Dr Jonathan Rohrer, at the University College of London. In the first phase of the project (GENFI-1, 2012-2015) no Spanish centre was included. In May 2015, the second phase of the multicentre project began (GENFI-2, May 2015-May 2020). Both British and philanthropic funds support the multicentre coordination platform, but each of the participating centres must finance the data acquisition costs generated in their centre. Due to our centre's previous experience in the study of this disease, and thanks to the funding obtained through this project, it was able to join this initiative, being the first Spanish centre of to participate. Through our membership

in GENFI, we also participate in the Frontotemporal Prevention Initiative, which also includes American and Australian centres with the aim of deepening knowledge in these diseases and designing and promoting clinical trials in this population.

According to the schedule, during the first semester of the project we adapted the GENFI clinical scales and cognitive batteries to our population and the validation of the acquisition of magnetic resonance imaging and other procedures needed to enter the study.

In September 2015, the first volunteer was included in our centre. Since then and until March 2019, 52 subjects affected by genetic frontotemporal dementia or first-degree relatives, and therefore at risk of developing it in the future, have been included in the GENFI-2 project in our centre (site 15). All of them have participated in the clinical, cognitive, biochemical biomarker and magnetic resonance studies. The data of these subjects have been sent to the coordinating centre and integrated with those from other GENFI centres and are part of phase 3 of data analysis (Data freeze, 3 May 2017).

In the multicentre study, retrospective data were collected on 3,020 individuals from 1,269 families with genetic frontotemporal dementia. The age of onset of the clinical symptoms of the disease varied depending on the gene affected. The mean age of onset in carriers of C9ORF72 is 58.6 years (SD 9.9), in carriers of MAPT mutation is 50.4 (9.6) and 61.4 (8.9) in carriers of mutations in GRN. The correlation between the age of actual onset and the expected age of onset in relation to the age of onset in the affected father or the average of onset in the family was evaluated. The strongest correlation was observed in carriers of MAPT mutations ($r = 0.55$, $p < 0.001$ and $r = 0.67$, $p < 0.001$). (Katrina M Dick et al., ICFTD, Munich, 2016, article in the drafting phase).

We investigated the levels of the light chain of neurofilaments (NfL) in cerebrospinal fluid in 174 subjects (48 controls, 40 presymptomatic carriers and 86 patients with mutations in the MAPT, GRN and C9orf72 genes) and serum NfL in 118 subjects (39 controls, 44 presymptomatic carriers, 35 patients). In 55 subjects, both CSF and serum were determined. The levels of NfL in the CSF in patients (median 6,762 pg / mL, interquartile range 3186-9309 pg / mL) were elevated compared to presymptomatic

carriers (804 pg / mL, 627-1173 pg/mL, P <0.001), allowing good discrimination between groups. The serum NfL levels correlated with the CSF levels (r = 0.87, P <0.001) and was also elevated in patients. The longitudinal samples in the converters showed an increase of three to four times in the NfL levels in CSF after the onset of symptoms. In addition, NfL levels in patients correlated with disease severity, brain atrophy, annualized brain atrophy rate and survival. These findings suggest that NfL levels in both serum and CSF have the potential to serve as a biomarker of the clinical onset of the disease and have a prognostic value in genetic FTD (Meeter et al., 2016).

In a multicentre study involving 30 centres, a complete genome association study (GWAS) was performed using the Illumina Human OmniExpress chip in 501 carriers of 114 different mutations in the GRN gene and 1173 matched controls. The age range of onset in patients was from 39 to 87 years. Significant signal was obtained with rs6966915 in TMEM106B (p = 7.89e -10, OR = 0.54 [0.45 - 0.66]) suggesting that this is a risk-modifying factor in carriers of mutations in GRN (Pottier et al., 2018).

Although the main objective of this project is to characterize the subjects with genetic frontotemporal dementia causing mutations in a multicentre manner, contributing data from our centre to the GENFI initiative and participating in the data analysis, generating and testing new hypotheses, some single-centre analyses have also been carried out that have generated interesting results:

We analyzed the neuropathological findings and immunohistochemical patterns of 9 patients with the P301L mutation who were donors of the Bank of Neurological Tissues, Biobank, Hospital Clínic-IDIBAPS. All the cases presented an astroglial, oligoglial and neuronal type 4R tauopathy with the presence of "mini-Pick" type bodies frequent in the dentate gyrus, suggesting that the P301L mutation presents a relatively homogeneous histological and immunohistochemical pattern (Borrego-Ecija et al., 2018).

We also analyzed 20 single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) ≥ 0.2 in the MAPT gene (17q21-22) in 20 subjects, of which 15 were carriers of the P301L mutation, belonging to 9 apparently unrelated families with frontotemporal dementia linked to the P301L mutation, all of them from the Baix Llobregat region. The analysis revealed that all carriers of the mutation had the same

haplotype for the analyzed SNPs, suggesting a common ancestral origin and therefore a founding effect of the P301L mutation in the MAPT gene in this geographical area. (Borrego-Ecija et al., 2018).

We studied the presence of ubiquitin / p62 positive inclusions in the granular cells of the cerebellum in 1800 brain from the Neurological Tissue Bank cohort of the Hospital Clínic-IDIBAPS regardless of their clinical or neuropathological phenotypes. These inclusions were found in 12 donors. In all cases, the presence of C9orf72 hexanucleotide expansion was confirmed by the genetic study, suggesting that the presence of ubiquitin / p62 positive inclusions in cerebellar granule cells is an specific marker for the detection of a pathogenic expansion in C9ORF72 (Ramos- Campoy O et al., 2018).

Additional cross-sectional data analyses are currently being carried out and the analysis of the longitudinal data obtained will be started soon.

2. Relevance and Possible Implications for Clinical care of the Results Obtained

As we mentioned in previous sections, this project's duration is 5 years, and therefore it does not end until May 2020. The foreseeable clinical implications are:

1. The clinical, neuroimaging, biochemical and neuropathological characterization will make it possible to improve the early detection of genetic cases and to establish a more accurate prognosis in patients and subjects at risk.

2. The characterization of the carriers of mutations causing frontotemporal genetic dementia and the quantification of the longitudinal changes in biomarkers, will make it possible to successfully design drug trials in this population, establishing accurate target engagement markers, markers of progression and optimal sample sizes.

3. Publications generated (only published papers are included here)

1: Premi E, Calhoun VD, Diano M, Gazzina S, Cosseddu M, Alberici A, Archetti S, Paternicò D, Gasparotti R, van Swieten J, Galimberti D, Sanchez-Valle R, Laforce R Jr, Moreno F, Synofzik M, Graff C, Masellis M, Tartaglia MC, Rowe J, Vandenberghe R, Finger E, Tagliavini F, de Mendonça A, Santana I, Butler C, Ducharme S, Gerhard A, Danek A, Levin J, Otto M, Frisoni G, Cappa S, Sorbi S, Padovani A, Rohrer JD, Borroni B; Genetic FTD Initiative, GENFI. The inner fluctuations of the brain in presymptomatic Frontotemporal Dementia: The chronnectome fingerprint. Neuroimage. 2019 Feb 1;189:645-654. doi: 10.1016/j.neuroimage.2019.01.080. [Epub ahead of print] PubMed PMID: 30716457.

2: 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. doi: 10.1093/jnen/nly047. PubMed PMID: 29889265.

3: Meeter LHH, Gendron TF, Sias AC, Jiskoot LC, Russo SP, Donker Kaat L, Papma JM, Panman JL, van der Ende EL, Dopper EG, Franzen S, Graff C, Boxer AL, Rosen HJ, Sanchez-Valle R, Galimberti D, Pijnenburg YAL, Benussi L, Ghidoni R, Borroni B, Laforce R Jr, Del Campo M, Teunissen CE, van Minkelen R, Rojas JC, Coppola G, Geschwind DH, Rademakers R, Karydas AM, Öijerstedt L, Scarpini E, Binetti G, Padovani A, Cash DM, Dick KM, Bocchetta M, Miller BL, Rohrer JD, Petrucelli L, van Swieten JC, Lee SE. Poly(GP), neurofilament and grey matter deficits in C9orf72 expansion carriers. Ann Clin Transl Neurol. 2018 Apr 6;5(5):583-597. doi: 10.1002/acn3.559. eCollection 2018 May. PubMed PMID: 29761121; PubMed CentralPMCID: PMC5945959.

4: Pottier C, Zhou X, Perkerson RB 3rd, Baker M, Jenkins GD, Serie DJ, Ghidoni R, Benussi L, Binetti G, López de Munain A, Zulaica M, Moreno F, Le Ber I, Pasquier F, Hannequin D, Sánchez-Valle R, Antonell A, Lladó A, Parsons TM, Finch NA, Finger EC, Lippa CF, Huey ED, Neumann M, Heutink P, Synofzik M, Wilke C, Rissman RA, Slawek

J, Sitek E, Johannsen P, Nielsen JE, Ren Y, van Blitterswijk M, DeJesus-Hernandez M, Christopher E, Murray ME, Bieniek KF, Evers BM, Ferrari C, Rollinson S, Richardson A, Scarpini E, Fumagalli GG, Padovani A, Hardy J, Momeni P, Ferrari R, Frangipane F, Maletta R, Anfossi M, Gallo M, Petrucelli L, Suh E, Lopez OL, Wong TH, van Rooij JGJ, Seelaar H, Mead S, Caselli RJ, Reiman EM, Noel Sabbagh M, Kjolby M, Nykjaer A, Karydas AM, Boxer AL, Grinberg LT, Grafman J, Spina S, Oblak A, Mesulam MM, Weintraub S, Geula C, Hodges JR, Piguet O, Brooks WS, Irwin DJ, Trojanowski JQ, Lee EB, Josephs KA, Parisi JE, Ertekin-Taner N, Knopman DS, Nacmias B, Piaceri I, Bagnoli S, Sorbi S, Gearing M, Glass J, Beach TG, Black SE, Masellis M, Rogaeva E, Vonsattel JP, Honig LS, Kofler J, Bruni AC, Snowden J, Mann D, Pickering-Brown S, Diehl-Schmid J, Winkelmann J, Galimberti D, Graff C, Öijerstedt L, Troakes C, Al-Sarraj S, Cruchaga C, Cairns NJ, Rohrer JD, Halliday GM, Kwok JB, van Swieten JC, White CL 3rd, Ghetti B, Murell JR,Mackenzie IRA, Hsiung GR, Borroni B, Rossi G, Tagliavini F, Wszolek ZK, Petersen RC, Bigio EH, Grossman M, Van Deerlin VM, Seeley WW, Miller BL, Graff-Radford NR, Boeve BF, Dickson DW, Biernacka JM, Rademakers R. Potential genetic modifiers of disease risk and age at onset in patients with frontotemporal lobar degeneration and GRN mutations: a genome-wide association study. Lancet Neurol. 2018Jun;17(6):548-558. doi: 10.1016/S1474-4422(18)30126-1. Epub 2018 Apr 30. PubMed PMID: 29724592; PubMed Central PMCID: PMC6237181.

5: 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. doi: 10.1159/000480077. Epub 2017 Sep 22. PubMed PMID:28934750.

6: Alcolea D, Vilaplana E, Suárez-Calvet M, Illán-Gala I, Blesa R, Clarimón J, Lladó A, Sánchez-Valle R, Molinuevo JL, García-Ribas G, Compta Y, Martí MJ, Piñol-Ripoll G, Amer-Ferrer G, Noguera A, García-Martín A, Fortea J, Lleó A. CSF sAPPβ, YKL-40, and neurofilament light in frontotemporal lobar degeneration. Neurology. 2017 Jul 11;89(2):178-188. doi: 10.1212/WNL.0000000000004088. Epub 2017 Jun 7. PubMed PMID: 28592456.

7: Meeter LH, Dopper EG, Jiskoot LC, Sanchez-Valle R, Graff C, Benussi L, Ghidoni R, Pijnenburg YA, Borroni B, Galimberti D, Laforce RJ, Masellis M, Vandenberghe R, Ber IL, Otto M, van Minkelen R, Papma JM, Rombouts SA, Balasa M, Öijerstedt L,Jelic V, Dick KM, Cash DM, Harding SR, Jorge Cardoso M, Ourselin S, Rossor MN, Padovani A, Scarpini E, Fenoglio C, Tartaglia MC, Lamari F, Barro C, Kuhle J, Rohrer JD, Teunissen CE, van Swieten JC. Neurofilament light chain: a biomarker for genetic frontotemporal dementia. Ann Clin Transl Neurol. 2016 Jul 1;3(8):623-36. doi: 10.1002/acn3.325. eCollection 2016 Aug. PubMed PMID: 27606344; PubMed Central PMCID: PMC4999594.

Scientific congresses

Demencia frontotemporal causada por la mutación P301L en el gen MAPT: revisión clínica y neuropatológica de 13 casos. Sergi Borrego, Joana Morgado, Oriol Grau, Ramón Reñé, Isabel Hernández, Mircea Balasa, Albert Lladó, Ellen Gelpi, Raquel Sánchez-Valle. LXVII Reunión Anual de la SEN 2015, Valencia, 15 November 2015.

Founder effect of P301L MAPT mutation in families with FTD in Baix Llobregat County (Barcelona, Spain). Leire Palencia-Madrid1; Sergi Borrego2, Oriol Grau-Rivera3, Ramón Reñé4, Isabel Hernández5, Consuelo Almenar6, Anna Antonell2, Albert Lladó2, Mircea Balasa2, Ellen Gelpi3, Marian M. de Pancorbo1; Raquel Sánchez-Valle2. 10th International Conference on Frontotemporal Dementias (Munich from 31/8/17 to 2/9/16)

Clinical and neuropathological features of Frontotemporal dementia caused by the P301L MAPT mutation in Baix Llobregat County (Barcelona, Spain). Sergi Borrego1, Joana Morgado2, Oriol Grau3, Ramón Reñé4, Isabel Hernández5, Consuelo Almenar6, Mircea Balasa1, Anna Antonell1, Albert Lladó1, Ellen Gelpi3, Raquel Sánchez-Valle1. 10th International Conference on Frontotemporal Dementias, (Munich del 31/8/17 al. 2/9/16)

P301L MAPT mutation causes globular glial tauopathy. Sergi Borrego1, Joana Morgado2, Oriol Grau3, Consuelo Almenar4, Mircea Balasa1, Albert Lladó1, Ellen Gelpi3, Raquel Sánchez-Valle1. 10th International Conference on Frontotemporal Dementias, (Munich from 31/8/16 to 2/9/16)

Utility of Ubiquitin/p62 aggregates screening in granular neurons of the cerebellar cortex in a Brain Bank: experience of the neurological tissue bank of the biobanc Hospital Clinic-IDIBAPS, Barcelona (Spain). R. Avila et al. 10th International Conference on Frontotemporal Dementias, (Munich from 31/8/16 to 2/9/16)

Symptom Onset in Genetic Frontotemporal Dementia. Katrina M Dick, et al. on behalf of the Frontotemporal dementia Prevention Initiative (FPI). 10th International Conference on Frontotemporal Dementias, (Munich from 31/8/v to 2/9/16)

Global initiative to identify genetic modifiers of disease onset and presentation in patients with progranulin mutations. Cyril Pottier et al. 10th International Conference on Frontotemporal Dementias, (Munich del from 31/8/16 to 2/9/16)

Increased neurofilament light chain correlates with decreased white matter integrity in presymptomatic and symptomatic granulin carriers J. L. Panman et al. 10th International Conference on Frontotemporal Dementias, (Munich from 31/8/16 to 2/9/16)

NOVEL P397S MAPT MUTATION CAUSING LATE ONSET SLOW PROGRESSIVE FRONTOTEMPORAL DEMENTIA. Sergi Borrego-Écija, Albert Lladó-Plarrumaní, Joan Puig Anton, Inmaculada Pericot, Carme Prat-Bravo, M Teresa Abellan, Jaume Olives, Neus Falgàs, Anna Antonell, Raquel Sánchez-Valle. 11th International Conference on Frontotemporal dementias. Sydney, November 11-14 2018.

DISTINCT AGE-RELATED CORTICAL THINNING IN ASYMPTOMATIC PROGRANULIN (GRN) MUTATION CARRIERS. Roser Sala-Llonch1, John van Swieten2, Barbara Borroni3, Fermin Moreno4, Mario Masellis5, Carmela Tartaglia6, Caroline Graff7, Daniela Galimberti8, Robert Laforce9, James Rowe10, Elizabeth Finger11, Rik Vandenberghe12, Fabrizio Tagliavini13, Alexandre de Mendonça14, Isabel Santana15, Matthis Synofzik16, Simon Ducharme17, Johannes Levin18, Adrian Danek18, Alex Gerhard19, Markus Otto20, Chris Butler21,Giovanni Frisoni22, Stefano Cappa22, Carolin Heller23, Rhian Convery23, Katrina M Moore23, Jonathan D Rohrer23, Raquel Sánchez-Valle1,24, on behalf of the Genetic FTD Initiative (GENFI). 11th International Conference on Frontotemporal dementias. Sydney, November 11-14 2018.