CATALAN NETWORK OF MULTIPLE SYSTEM ATROPHY: BIOMARKERS AND PATHOPHYSIOLOGY

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1. Project Summary

Objectives

- To create a Catalan clinical registry of MSA patients including demographic, epidemiological and clinical data.
- To create a biorepository of different biological samples (DNA, CSF, serum, plasma, RNA and fibroblasts) from MSA patients for further research purposes,
- To identify interactions of specific proteins and microRNAs with transcripts of a-synuclein (in particular with UTRs) by in silico and in vitro analysis, and investigate their role in synuclein pathology.
- To find differential expression of microRNA and proteins interacting with synuclein transcripts in MSA, and other previous candidate proteins, that could be potentially used as progression and diagnostic biomarkers.

Methodology

80-90 MSA patients extensively clinically studied in different Catalan centers will be included in the project. All data will be entered in an on-line platform by the Barcelona center. Different biomarkers will be collected from all patients and stored in a biorepository: DNA, cerebrospinal fluid (CSF), blood serum, plasma, and cultured fibroblasts from skin biopsies. In silico analyses and protein arrays will be performed to identify proteins and microRNAs interacting with a-synuclein RNA. Complementary techniques (e.g. CSF analyses) will be used to study these candidate proteins and microRNAs in MSA.

Work Plan

- Semester 1: Generation of a computer-based MSA patient clinical registry.
- Semester 1-5: Recruitment of 90 MSA patients (30 during the first two semesters): clinical analysis, ad biosamples of all 90 MSA patients; creation of biorepository specific for MSA.
- Semester 3-6: Identification of RNA binding proteins to synuclein UTR/CSF and blood biomarker studies, and publication of results.

2. Objectives Achieved

During these 3 years we have:

- Generated a computer-based MSA patient clinical registry.
- Recruited 80 patients affected by MSA.
- Collected exhaustive clinical information and completed neuropsychological studies with a total of 208 medical consultations (including baseline and follow-up consultations with a total of 80 patients).
- Collected 191 biosamples of plasma, serum and RNA; 139 biosamples with urine, 56 of CSF and 46 Skin biopsy.

As mentioned previously, we recruited 80 MSA patients, 39 were females and 41 were males. The mean age at baseline consultation was 63.5 years, in the 42-83 age range. The mean age of onset of the first motor symptom is 58, (37-76 age range), mean disease duration is 5 years (63 months). Forty-four (55%) cases had MSA_P, and 36 (45%) MSA-C, all of them being probable. We collected: 46 skin biopsy samples, 56 CSF samples, 191 serum, blood and RNA samples, as well as 142 of urine. CSF and skin biopsies samples were collected only at the baseline consultations (T0), but during the follow-up consultations every 6 months we also collected blood and urine samples (T1, T2, T3, T4). All materials were stored in a biorepository in Hospital Clinic de Barcelona. For group comparisons we have also recruited 6 healthy control patients (3 F: 3M), mean age 64 years with no neurological disorders or other serious illnesses. Controls underwent the same clinical evaluation and neuropsychological evaluation as MSA patients. We have collected blood with serum, plasma and RNA from all 6 controls, and 4 underwent skin biopsy and 3 CSF extractions as well.

Sex(n=80)	Male: **41**	Female: **39**
Age at basal visit	63.5	
MSA Subtype(n=80)	MSA-P (Parkinsonian)	44
	MSA-C (Cerebellar)	36

		T0	T1 (6m)	T2 (12m)	T3 (18m)	T4 (24m)
Included		80 + 6C	58	40	23	7
		T0	T1	T2	T3	T4
Clinical Evaluation		80+ 6C	58	40	23	7
Neuropsychiatric evaluation		76+ 6C	55	36	20	5
Collected Biosamples	Serum/plasma/RNA	80 +6 C	54	35	17	5
	Urine	71+ 6C	37	24	8	2
	LCR	56 + 3C	-	-		
	Fibroblasts (Skin biopsy)	46 + 4C	-	-		

3. Relevance and possible clinical applicability of the final results

Currently the diagnosis of MSA is clinical. Cases are diagnosed following consensus guidelines that establish a possible or probable diagnosis. Definite diagnosis is only possible at autopsy. The consensus criteria are useful but are not always able to diagnose the disease at early stages, and predictive values have not been established for individual prognosis. Furthermore, MSA is a rare disease with an incidence of 0.6/100,000 and a prevalence of 4/100,000, which means that most studies are small and significant results are difficult to reproduce.

Thanks to the data collected during these last three years we have an appropriate number of subjects to fully describe the clinical aspects of the disease with longitudinal prospective data that can help update clinical guidelines for a more accurate diagnosis, even in early cases. Diagnosis will become relevant as new therapies are being developed that may modify the course of the disease, such as immunotherapy. MSA is often misdiagnosed as Parkinson disease, and vice versa, which may confound results from clinical trials. We hope our clinical data will be valuable to establish better clinical assessments and relevant clinical differentiation from other diseases.

In addition, our biosample collection will be useful for biomarker discovery. Understanding the clinical profile of the disease is important, but relying exclusively on clinical data for diagnosis and disease monitoring is insufficient. We have ongoing research projects using blood samples to study differential proteomic and transcriptomic profiles, as well as CSF in search of specific inflammatory or metabolic

features of the disease. MSA is in need of objective diagnostic biomarkers as mentioned earlier to aid in a more accurate or even definite diagnosis. However, progression biomarkers are also important to predict prognosis and evaluate the effect of possible therapies to come. Our follow-up collection of blood and urine samples can be used in search of progression biomarkers as well, evaluating the changes of specific molecules in the same patient at 6-month intervals.

Finally, by studying transcriptomic, inflammatory and molecular processes we shed light on the pathophysiology of the disease, which is still widely unknown. By understanding the pathological mechanisms of the disease, new therapeutic approaches can be tested.

Moreover, with this broad cohort of MSA patients other centers may be interested in collaborating with us for international multicenter research projects. We have already been contacted by European and American centers looking forward to sharing clinical data and biosamples with us.

4. Publications, communications and training of personnel derived from this research

Posters

The Catalan multiple system atrophy-registry (CMSAR). Antonelli F, Muñoz E, Pagonabarraga J, Hernández-Vara J, Bayes A, Oriol de Fabregues, Valldeoriola F, Tolosa E, Compta Y, Ezquerra M, Fernandez R, Calopa M, Jauma S, Pujol M, Puente, V, Cámara A, Planellas L, Martí, MJ Abstract accepted at the **5th International Congress on Multiple System Atrophy;** Poster Number: 132; Salerno, April 22-23, 2016.

The Catalan multiple system atrophy-registry (CMSAR). Antonelli F, Muñoz E, Pagonabarraga J, Hernández-Vara J, Bayes A, Oriol de Fabregues, Valldeoriola F, Tolosa E, Compta Y, Ezquerra M, Fernandez R, Calopa M, Jauma S, Pujol M, Puente, V, Cámara A, Planellas L, Martí, MJ Abstract accepted at the **20th International Congress of The International Parkinson and Movement Disorder Society (MDS);** Poster Number: 151; Berlin, June 19-23, 2016.

Y. Compta, F. Antonelli, M. Fernandez, P. Bravo, M. Soto, A. Camara, D.MGiraldo, M.J. Marti, On behalf- of Catalan-MSA-Registry Group. Cerebrospinal Fluid Levels of Coenzyme Q10 are Reduced in Multiple System Atrophy [abstract]. *Mov Disord.* 2017; 32 (suppl 2). accepted at the **21st International Congress of the International Parkinson and Movement Disorder Society (MDS);** Poster Number: 172; Vancouver, June 4-8, 2017. http://www.mdsabstracts.org/abstract/cerebrospinal-fluid-levels-of-coenzyme-q10-are-reduced-inmultiple-system-atrophy/.

D. Giraldo, Y. Compta, F. Antonelli, E. Muñoz, A. Camara, J. Pagonabarraga, O. de Fabregués, J. Hernández-Vara, F. Valldeoriola, E. Tolosa, M.C. Pont, S. Jauma, A. Bayes, N. Caballol, M. Calopa, P. Pastor, L. Planellás, M. Pujol, V. Puente, A. Avila, M.J. Martí. Cognitive Impairment in MSA Patients from the Catalan Multiple System Atrophy Registry (CMSAR) [abstract]. *Mov Disord.* 2017; 32 (suppl 2). accepted at the **21st International Congress of The International Parkinson and Movement Disorder Society (MDS);** Poster Number: 166; Vancouver, June 4-8, 2017. http://www.mdsabstracts.org/abstract/cognitive-impairment-in-msa-patients-from-the-catalanmultiple-system-atrophy-registry-cmsar/.

Y. Compta, S. Dias, M. Pulido-Salgado, D. Giraldo, A. Pérez-Soriano, M. Fernández, A. Cámara, P. Bravo, E. Muñoz, J. Saura, MJ. Marti. Cerebrospinal fluid levels of cytokines in multiple system atrophy: A cross-sectional study of the Catalan msa registry (CMSAR) [abstract]. Mov Disord. 2018; 33 (suppl 2). http://www.mdsabstracts.org/abstract/cerebrospinal-fluid-levels-of-cytokines-in-multiple-system-atrophy-a-cross-sectional-study-of-the-atalan-msa-registry-cmsar/.

DM. Giraldo, A. Pérez Soriano, J. Rios Guillermo, E. Muñoz, Y. Compta, J. Pagonabarraga, F. Valldeoriola, J. Hernández-Vara, S. Jauma Classen, V. Puente, C. Pont, N. Caballol, E. Tolosa, A. Bayes, J. Campdelacreu, O. Fabregues, A. Avila, M. Calopa, C. Gaig, N. Fabregat, P. Pastor, M. Aguilar, M. Pujol, L. Planellas, A. Camara, M.J. Marti. Non-motor symptoms in patients from the Catalonian Multiple System Atrophy Registry (CMSAR) [abstract]. Mov Disord. 2018; 33 (suppl 2). http://www.mdsabstracts.org/abstract/non-motor-symptoms-in-patients-from-the-catalonian-multiple-system-atrophy-registry-cmsar/.

A. Perez-Soriano, DM. Giraldo, J. Rios-Guillermo, E. Muñoz, Y. Compta, MJ. Marti, On-behalf-of-Catalonian-MSA-Registry. The Catalonian Multiple System Atrophy Registry (CMSAR): The motor features and their impact on disability status in MSA patients [abstract]. Mov Disord. 2018; 33 (suppl 2). http://www.mdsabstracts.org/abstract/the-catalonian-multiple-system-atrophy-registry-cmsar-the-motor-features-and-their-impact-on-disability-status-in-msa-patients

Oral communications in XXI and XXII Reunió Annual de la Societat Catalana de Neurologia May 2017 and 2018, Barcelona:

Cerebrospinal Fluid Levels of Coenzyme Q10 are Reduced in Multiple System Atrophy. Y. Compta, F. Antonelli, M. Fernandez, P. Bravo, M. Soto, A. Camara, D.M. Giraldo, M.J. Marti, On behalf- of Catalan-MSA-Registry Group.

Cognitive Impairment in MSA Patients from the Catalan Multiple System Atrophy Registry (CMSAR). D. Giraldo, Y. Compta, F. Antonelli, E. Muñoz, A. Camara, J. Pagonabarraga, O. de Fabregués, J. Hernández-Vara, F. Valldeoriola, E. Tolosa, M.C. Pont, S. Jauma, A. Bayes, N. Caballol, M. Calopa, P. Pastor, L. Planellás, M. Pujol, V. Puente, A. Avila, M.J. Martí.

A. Perez-Soriano, DM. Giraldo, J. Rios-Guillermo, E. Muñoz, Y. Compta, MJ. Marti, On-behalf-of-Catalonian-MSA-Registry. The Catalonian Multiple System Atrophy Registry (CMSAR): The motor features and their impact on disability status in MSA patients. D.M. Giraldo, A. Pérez Soriano, J. Rios Guillermo, E. Muñoz, Y. Compta, J. Pagonabarraga, F. Valldeoriola, J. Hernández-Vara, S. Jauma Classen, V. Puente, C. Pont, N. Caballol, E. Tolosa, A. Bayes, J. Campdelacreu, O. Fabregues, A. Avila, M. Calopa, C. Gaig, N. Fabregat, P. Pastor, M. Aguilar, M. Pujol, L. Planellas, A. Camara, M.J. Marti. Non-motor symptoms in patients from the Catalonian Multiple System Atrophy Registry (CMSAR)

Final Master & Thesis Work:

Cerebrospinal fluid inflammation biomarkers in multiple system atrophy. Sara Dias. June 16, 2017.

Cognitive and Neuropsychiatric impairment in multiple system atrophy. Darly Milena Giraldo. June 16, 2017.

Alexandra Perez Soriano has obtained a grant for the thesis titled **PhD4MD fellowship** and will work on a collaboration projected between two research centres "Multiple System Atrophy: Synuclein Regulation and Biomarkers Discovery" (Dr. Gian Tartaglia, CRG and Dr. Maria José Martí, IDIBAPS) from October 2017 to 2020.

Published articles and articles in progress:

Marchese, D., Botta-Orfila, T., Cirillo, D., Rodriguez, J. A., Livi, C. M., Fernández-Santiago, R., Ezquerra, M., Martí, M. J., Bechara, E., Tartaglia, G. G., Catalan MSA Registry (CMSAR) (2017). Discovering the 3' UTR-mediated regulation of alpha-synuclein. *Nucleic acids research*, *45*(22), 12888-12903.

Yaroslau Compta, Darly M. Giraldo, Esteban Muñoz, Francesca Antonelli, Manel Fernández, Paloma Bravo, Marta Soto, Ana Cámara, Ferran Torres, María José Martí, Asunción Ávila, Àngels Bayés, Teresa Botta-Orfila, Núria Caballol, Matilde Calopa, Jaume Campdelacreu, Mario Ezquerra, Oriol de Fàbregues, Rubén Fernández-Santiago, Jorge Hernández-Vara, Serge Jaumà, Domenica Marchese, Javier Pagonabarraga, Pau Pastor, Lluís Planellas, Claustre Pont-Sunyer, Víctor Puente, Montserrat Pujol, Josep Saura, Gian Gaetano Tartaglia, Eduard Tolosa, Francesc Valldeoriola. Cerebrospinal fluid levels of coenzyme Q10 are reduced in multiple system atrophy, Parkinsonism & Related Disorders, Volume 46, 2018, Pages 16-23 ISSN 1353-8020, https://doi.org/10.1016/j.parkreldis.2017.10.010.

Abos, Alexandra; Baggio, Hugo; Segura, Barbara; Campabadal, Anna; Uribe, Carme; Milena, Darly; Pérez, Alexandra; Muñoz, Esteban; Compta, Yaroslau; Junque, Carme; Marti, Maria Jose. Probabilistic tractography for the characterization of white matter abnormalities and discrimination of multiple system atrophy from Parkinson's disease. Manuscript number: HBM-18-1231. *Pending revision and approval* by Human Brain Mapping Editorial Office.

Yaroslau Compta, Sara Dias, Darly M. Giraldo, Alexandra Pérez-Soriano, Esteban Muñoz, Josep Saura, Manel Fernández, Paloma Bravo, Ana Cámara, Marta Pulido-Salgado, Cèlia Painous and María José Martí* on behalf of the CMSAR consortium. Cerebrospinal Cytokines levels as biomarkers of multiple system atrophy: A cross-sectional study of The Catalan MSA Registry. (CMSAR). *Parkinsonism & Related Disorders (in press)*.

Manuscripts in drafting process and ongoing research projects:

NON-MOTOR SYMPTOMS IN THE CATALONIAN MULTIPLE SYSTEM ATROPHY REGISTRY (CMSAR) by A. Perez-Soriano, DM. Giraldo, E. Muñoz, Y. Compta, MJ. Marti, On-behalf-of-Catalonian-MSA-Registry.

Transcriptomics and biomarker discovery by Alexandra Pérez Soriano, Magdalena Arnal Segura, Manel Fernández, Teresa Botta-Orfila, Mario Ezquerra, Rubén Fernández-Santiago, Gian Gaetano Tartaglia, Y. Compta, MJ. Marti.

MiRNA differential expression between MSA and controls discovery by Alexandra Pérez Soriano, Magdalena Arnal Segura, Manel Fernández, Teresa Botta-Orfila, Mario Ezquerra, Rubén Fernández-Santiago, Gian Gaetano Tartaglia, Y. Compta, MJ. Marti.