MRI BRAIN CONNECTOMICS AND METABOLIC IMAGING IN THE TGF344-AD RAT: A NEW APPROACH FOR THE STUDY OF ALZHEIMER'S DISEASE

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1. Summary of the project

Alzheimer's disease (AD) is a progressive age-related neurodegenerative disease that has become a major healthcare concern due to its vast prevalence. Drug development against AD has been hindered by the lack of translationality in preclinical research, underscoring the need for a shift in the paradigm to study this disorder. We propose a novel and integrative way of studying AD with longitudinal and multimodal magnetic resonance imaging (MRI) and spectroscopy to obtain brain functional and structural connectivity and metabolic data in a model of AD. The TgF344-AD rat is a new and promising transgenic rat model of AD which manifests the full array of AD pathology, age-dependent cerebral amyloidosis that precedes tauopathy, gliosis, apoptotic loss of neurons in the cerebral cortex and hippocampus, and cognitive disturbance.

The main objectives of this project were: 1) to improve the following MRI acquisition protocols for its use in experimental rat models: resting state functional MRI (RSS-fMRI), diffusion weighted imaging (DWI) and 1H magnetic resonance spectroscopy (1H-MRS); 2) to perform a multimodal and longitudinal (every 3 months, from 12 to 60 weeks of age) MRI study in TgF344-AD rats and their wild-type littermates to specially investigate the very initial phases of the pathology by RSS-fMRI functional connectomics, DWI structural connectomics and 1H-MRS metabolic imaging; and 3) to correlate the information obtained from both structural and functional connectomics and the metabolic study with the cognitive results from a working memory task performed by the same animals, to define non-invasive early markers of the disease.

2. Results

From a technical point of view, we investigated the influence of voxel geometry on diffusion weighted imaging (DWI) analysis, comparing different acquisition orientations as well as isometric and anisometric voxels. The acquisition direction with respect to the main magnetic field orientation affected the diffusion results. When the acquisition slice-encoding direction was not aligned with the main magnetic field, there were more artifacts and a lower signal-to-noise ratio that led to less anisotropic tensors (lower fractional anisotropic values), producing poorer quality results. It also elicited differences in tract reconstruction and in different graph metric values describing the

brain networks. Our results highlight the importance of taking into account the geometric aspects of acquisitions, especially when comparing diffusion data acquired using different geometries. *Tudela R, et al. 2017. PLoS ONE 12(1): e0170703.*

Proton MR spectroscopic imaging (MRSI) can provide a variety of "molecular images" from animal models of human disease, which are useful for different research purposes. After optimizing the procedure we wrote a "Hands on" chapter describing a protocol for in vivo acquisition and analysis of MRSI data from the rodent brain. *Simoes et al., 2018 https://doi.org/10.1007/978-1-4939-7531-0_12.*

Our longitudinal, multimodal study performed in TgF344-AD rats generated an enormous amount of data. For the initial phase of the project, resembling the silent or preclinical phase of AD, we found alterations in global and regional structural networks together with regional differences in functional networks of young adult TgF344-AD rats, at as early a period as 5 months of age. At a global level, structural networks showed lower integration and segregation in transgenic than in control rats, pointing to a different pattern of anatomical connections in subjects developing AD. Structural connectivity differences did not lead to changes in global functional metrics, probably because of changes induced in the resting-state connectivity by the long cognitive training phase the animals underwent before MRI scanning. Differences in the functional or structural network properties were shown in several regions related to memory or reward circuit, known to be altered in patients with AD or MCI. Therefore, this study suggests a pattern of alteration in the brain network with consequences in cognition already present at very early stages of the disease, when most of the pathological hallmarks have not yet been detected. *Muñoz-Moreno et al. 2018. Alzheimer's Research & Therapy 2018, 10:16.*

The follow-up study revealed that these differences were also observed later in time, together with a different evolution of the structural metrics. While aging had no significant effect on the evolution of network metrics in the control group, it significantly affected metrics in the transgenic animals. In this group, metrics increased linearly with age, with a sudden decrease in the metric values at the last time point. Although there were no significant differences in either the functional networks or cognitive performance among either the experimental groups or the different ages studied the relationship between cognition and connectivity was different in each

group. While working memory results were influenced by the structural brain network in transgenic animals, functional network had a major effect on the performance of control subjects.

In addition, we found longitudinal changes in functional connectivity in TgF344-AD rats in somatosensory, sensorimotor and default mode resting state networks and their influence in the cognitive outcome. Moreover, TgF344-AD rats showed alterations in the metabolite profile of hippocampus and striatum associated to a decrease of Tau, especially pronounced at the earlier stages, decreases of Glu, NAA and NAAt and an increase of Gln.

Altogether, the results confirm findings observed in human populations at risk of AD and provide new insights in the evolution of brain networks affected by the disease. Results support the idea of AD as a continuum and the disconnection hypothesis, pointing to different organization of structural brain network whose consequences in cognitive impairment could be compensated by functional connectivity until advanced stages of AD, suggesting the influence of cognitive reserve on the preservation of functional abilities in AD subjects.

3. Relevance with possible future implications

We have demonstrated that in an early phase of the disease where neurons are preserved, no beta amyloid plaques are being accumulated yet, nor is any hyperphosphorylation of Tau protein observed, and structural and functional connectivity alterations can be observed in TgF344AD rats. Some of these alterations remain stable during the progression of the pathology; others evolve with time differently from wild-type animals.

In conclusion, the connectivity analysis of TgF344AD rats not only replicates results observed in human populations at risk of AD, but it allows characterizing earlier stages of the disease and follow up its progression to identify breaking points in network evolution. These results point to the potential of MRI-based connectomics as early biomarker of AD.

Our results support AD as a continuum and the disconnection hypothesis, pointing to a different organization of structural brain network whose consequences in cognitive impairment can be compensated by functional connectivity until advanced stages of the disease.

An important although preliminary finding of this project suggests that intensive cognitive stimulation could prevent the functional cognitive decline observed in this animal model of AD despite the structural alterations observed.

4. Literature generated

Tudela R; Muñoz-Moreno E; López-Gil X; Soria G. Effects of orientation and anisometry of magnetic resonance imaging acquisitions on diffusion tensor imaging and structural connectomes. PLOS ONE. ISSN/ISBN: 1932-6203. 01-01-2017, vol.: 12 nº: 1. pg: ARTN e0170703 -. DOI:10.1371/journal.pone.0170703.

Simões R, Muñoz-Moreno E, Tudela R, Soria G. ^{1}H spectroscopic imaging of the rodent brain. METHODS IN MOLECULAR BIOLOGY. ISSN/ISBN: 1940-6029. 01-01-2018, vol.: 1718 pg: 189 -202. DOI:10.1007/978-1-4939-7531-0_12.

Muñoz-Moreno E, Tudela R, López-Gil X, Soria G. Early brain connectivity alterations and cognitive impairment in a rat model of Alzheimer's disease. ALZHEIMER'S RESEARCH AND THERAPY. ISSN/ISBN: 17589193. 02-07-2018, vol.: 10 nº: 1. pg: 16 -16. DOI:10.1186/s13195-018-0346-2.

Under review in Translational Neurodegeneration - TNEU-D-19-00032:

Brain connectivity during Alzheimer’s disease progression and its cognitive impact in a transgenic rat model. Emma Muñoz-Moreno, Raúl Tudela, Xavier López-Gil, Guadalupe Soria

In preparation:

Connectivity evolution in functional resting state networks of the TgF344-AD rat model of Alzheimer’s disease. Raúl Tudela, Emma Muñoz-Moreno, Roser Sala-Llonch, Xavier López-Gil and Guadalupe Soria.

Brain metabolic changes associated to the progression of Alzheimer's disease in a transgenic rat model. Emma Muñoz-Moreno, Rui Vasco Simoes, Raúl Tudela, Guadalupe Soria.

Correlation between neurite orientation dispersion and density imaging (NODDI) and plaque quantification in a transgenic model of Alzheimer’s disease. Laia Sitjà, Emma Muñoz-Moreno, Laura Molina, Raúl Tudela, Xavier López-Gil, Guadalupe Soria