

MIND FACE

Decreased NAA concentration in presymptomatic *C9orf72*-HRE mutation carriers in the anterior cingulate cortex using 7T-MRS

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Aims, research questions and working hypothesis

Frontotemporal lobar degeneration (FTLD) is the second leading cause of early-onset dementia, characterized by frontal and/or temporal lobe atrophy and protein accumulation (tau or TDP-43) in 95% of cases. While most cases are sporadic, some result from mutations in *microtubule associated protein tau* (*MAPT*), *progranulin* (*GRN*), or *Chromosome 9 open reading frame 72* (*C9orf72*) repeat expansion¹. The disease mechanism remains unclear, but neuroinflammation, involving activated microglia, astrocytes, and increased proinflammatory cytokines, is a recognized component in both FTLD-tau and FTLD-TDP². Understanding neuroinflammation's role and timing is essential to assess anti-inflammatory therapy potential.

Proton Magnetic Resonance Spectroscopy (¹H MRS) measures brain metabolite concentrations, providing insight into cell-specific intracellular processes. Neuronal markers include N-acetyl-aspartate (tNAA) and glutamate (Glu), while glial markers are myo-inositol (ml) and choline (Cho). Creatine (tCr) serves as a reference^{3, 4}. Elevated ml and Cho levels suggest glial activation and neuroinflammation, while decreased tNAA and Glu indicate neuronal atrophy^{3, 5}. MRS has shown that ml levels are increased, before reduction of tNAA levels, suggesting that neuroinflammation often precedes neuronal loss, as seen in presymptomatic *MAPT* mutation carriers⁶⁻¹¹. However, these results come from a very small studies with on 1.5T and 3T or without specified pathologies or mutations.

MRS using a 7T MRI could detect early neuroinflammatory changes in FTLD, due to improved spectral resolution and signal-to-noise ratio. However, it has not yet been applied to *GRN* or *C9orf72* mutation carriers or sporadic cases. This study uses MRS on 7T MRI in (pre)symptomatic participants with *GRN*, *MAPT*, and *C9orf72* mutation carriers, sporadic cases, and healthy controls to explore these changes.

Means/methods implemented by the consortium

Subjects: We included 55 participants: 12 symptomatic carriers, 26 presymptomatic carriers, and 17 healthy controls. Mutation status of the carriers was confirmed by genetic testing. Study protocols were approved by the institutional review board and written informed consent obtained. Inclusion and data acquisition is still ongoing.

MRI setup: Experiments were conducted on a 7T Philips Achieva MRI system (Best, The Netherlands) using a quadrature transmit and 32-channel receive head coil (Nova Medical, Wilmington, MA, USA).

In vivo MR protocol and post-processing: A 3D-T1w MPRAGE (TR/TE: 6.2/2.7 ms, resolution: 0.6x0.6x0.6mm³, scantime: 08:57) acquisition was used for positioning of the volume-of interest (VOI). T1-w image was segmented using SPM, and partial volumes were calculated using FSL. Single-volume spectroscopy (SVS) data were acquired using STEAM sequence (TR/TE/TM: 600/17/25 ms, acquisition time: 05:36, number of averages: 48) from a 35x20x15mm³ volume-of-interest (VOI) positioned in the right or left lateral anterior cingulate cortex. SVS data were reconstructed using MATLAB and LCModel¹² with a TE-matched basis-set. We measured levels normalized to tCr of the following metabolites: tNAA, NAA, tCho, ml, and Glu.

Statistical analysis: The statistical analysis was done on the healthy controls (n=17) and presymptomatic C9 carriers (n=14), a subset of the data due to the group sizes. Age, gender, percentage white matter and grey matter were compared between the two groups using a student's t-test. Metabolite concentrations, i.e., NAA/tCr, tCho/tCr, ml/tCr, Glu/tCr, per groups were tested using linear mixed models corrected for age and gender. tNAA was not included, as the data was not normally distributed. P-values were corrected for multiple testing.

What are the outcomes of the project?

The results presented here are preliminary results as the inclusion is still ongoing. There were no differences in the demographics or in tissue volume fractions between the presymptomatic C9 carriers and the healthy controls. Linear mixed models showed a significant difference in NAA/tCr concentration between the presymptomatic C9-group and the healthy controls (p = 0.00318). No differences were found for the other

metabolites ($p > 0.05$). There seems to be an added effect of age for the ratio of NAA/tCr ($p = 0.00826$) and Glu/tCr ($p = 0.00459$).

Significance and impact of the work on the field

In this preliminary analysis, we found a difference in NAA/tCr ratio in presymptomatic *C9orf72* mutation carriers compared to healthy controls. The decrease in NAA, a neuronal integrity marker, aligns with previous research indicating brain atrophy in presymptomatic *C9orf72*-HRE and the hypothesis that presymptomatic *C9* carriers have smaller average brain volumes^{15,16}. Additionally, we found an added age effect for NAA/tCr and Glu/tCr. We are continuing data acquisition for more elaborate data analysis.

Next steps and future challenges

As the inclusion of the study is still ongoing, we will continue data acquisition and expand the statistical analysis to the other mutation groups and the symptomatic participants.

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