INTEGRATIVE CHARACTERIZATION OF A RODENT ALZHEIMER'S DISEASE MODEL

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BACKGROUND

➔ Alzheimer's Disease (AD) is a severe neurodegenerative disorder with progressive loss of memory and cognitive functions.

➔ To investigate Alzheimer-related pathophysiology, several transgenic mouse and rat lines have been established in recent years.

➔ Despite their general applicability in basic and applied research, quantitative tools to monitor pathophysiology as well as associated rewiring of metabolic pathways on a systemic level are lacking.

AIM

➔ Use an integrative approach – behavioral tests, immuno-fluorescence and untargeted nuclear magnetic resonance (NMR) based metabolic phenotyping to get a better understanding of (patho-) physiological alterations in complex biological networks involved in AD.

MATERIALS AND METHODS

➔ Tg4-42 and Wild-type mice of different ages were tested in the Morris water maze test to receive read-outs of the disease related to spatial learning and memory.

➔Neuroinflammation and plaque load were analyzed by immunofluorescent labeling with GFAP, Iba-1 and Aβ1-42 antibodies, respectively.

➔Untargeted NMR spectroscopy to monitor perturbations in a large pool of metabolites

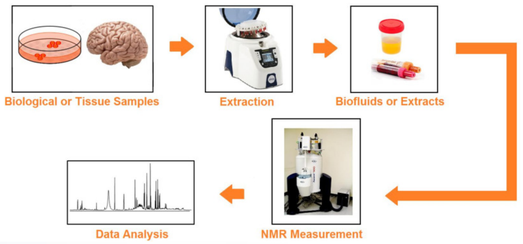

Figure 1: NMR-based metabolic phenotyping
To define a metabolite biomarker panel, metabolites were extracted from brain tissues like cortex, hippocampus and restbrain. For the tissues a soft homogenization with a Precellys system was performed. Serum or other biofluids can be used directly.
The measurement was done with a 600 MHz NMR spectrometer using an untargeted approach.
As the last step, data analysis was prepared using statistical tools (PCA, OPLS-DA).

RESULTS

➔ Behavioral test

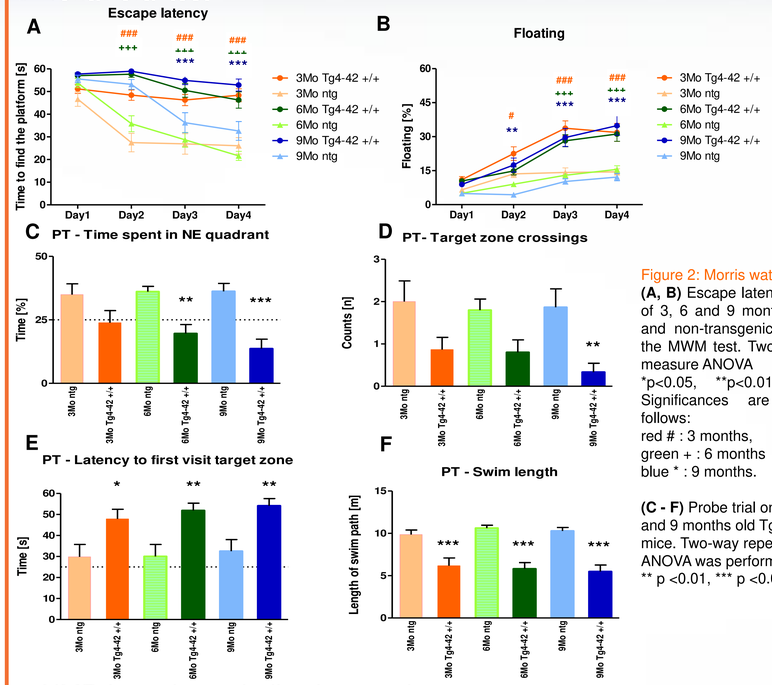

Figure 2: Morris water maze test
(A, B) Escape latency and floating of 3, 6 and 9 months old Tg4-42 and non-transgenic (ntg) mice in the MWM test. Two-way repeated measure ANOVA
*p<0.05, **p<0.01, ***p<0.001; Significances are labeled as follows:
red # : 3 months,
green + : 6 months
blue * : 9 months.

(C - F) Probe trial on day 5 of 3, 6 and 9 months old Tg4-42 and ntg mice. Two-way repeated measure ANOVA was performed (* p <0.05, ** p <0.01, *** p <0.001).

➔ Immunofluorescent labeling

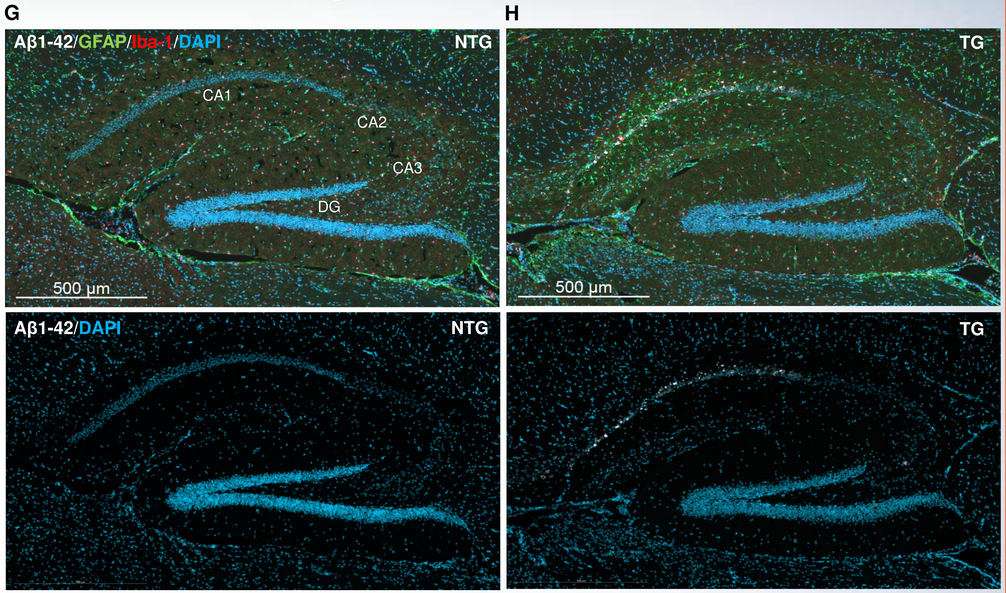

Figure 3: Immunofluorescent labeling
Brain tissue was labeled with amyloid beta 1-42, GFAP to determine astrogliosis, Iba-1 for microgliosis and DAPI nuclear staining in non transgenic **(G)** and Tg4-42 transgenic mice **(H)**. Amyloid beta was found only in Tg4-42 transgenic mice mainly in the CA1 region of the hippocampus. Also astrogliosis was found in this region of the Tg4-42 transgenic animals. Furthermore the CA1 cell layer is thinner in Tg4-42 transgenic mice
(CA = cornu ammonis; DG = dentatus gyrus).

➔ NMR-based metabolic phenotyping

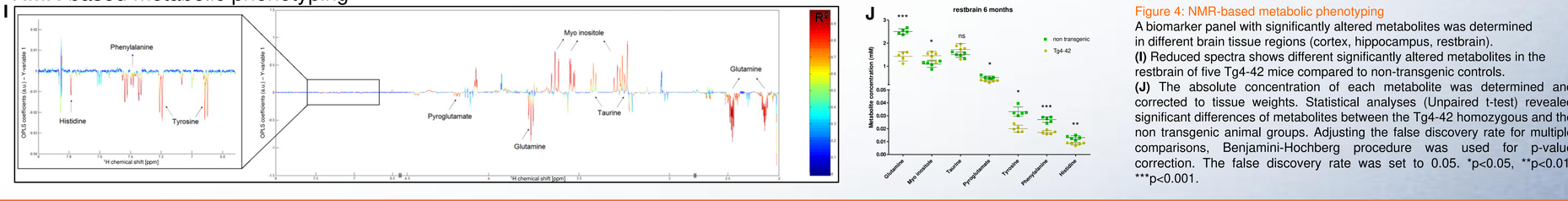

Figure 4: NMR-based metabolic phenotyping
A biomarker panel with significantly altered metabolites was determined in different brain tissue regions (cortex, hippocampus, restbrain).
(I) Reduced spectra shows different significantly altered metabolites in the restbrain of five Tg4-42 mice compared to non-transgenic controls.
(J) The absolute concentration of each metabolite was determined and corrected to tissue weights. Statistical analyses (Unpaired t-test) revealed significant differences of metabolites between the Tg4-42 homozygous and the non transgenic animal groups. Adjusting the false discovery rate for multiple comparisons, Benjamini-Hochberg procedure was used for p-value correction. The false discovery rate was set to 0.05. *p<0.05, **p<0.01, ***p<0.001.

CONCLUSION

➔ The combination of different methods is important to link changes in biomarkers and the associated dysregulation of metabolic pathways with changes in neuropathology and behavior.

➔ This integrative approach (behavioral studies, immunofluorescence, NMR-based metabolic phenotyping) not only contributes to the understanding of this devastating neurodegenerative disease and the related pathophysiological processes on a systemic level, but sets the base for a wide range of biomedical applications. It can be easily extended to other tissues, matrices, or disease models and translated across species.

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