Quantitative Diffusion, Perfusion and T_2 brain MRI of the α-syntrophin knockout mouse in the resting state

C. Brekken[1], A. Håberg[1], T. Pavlin[1], P. Goa[1,2], A. Bjørnerud[3], O. Haraldseth[1,2], O. Ottersen[4], and E. Nagelhus[4]

[1]Circulation and Medical Imaging, NTNU, Trondheim, Norway, [2]Medical Imaging, St.Olavs Hospital HF, Trondheim, Norway, [3]The National Hospital, Oslo, Norway, [4]University of Oslo, Oslo, Norway

INTRODUCTION

The predominant water channel protein in brain, aquaporin-4 (AQP4), is concentrated in the astrocyte end-feet membranes adjacent to blood vessels in neocortex and cerebellum by association with alpha-syntrophin (α-Syn) protein (Ref. 1). To investigate whether depletion of α-syn (~90%) affects cortical water diffusion, cerebral perfusion and brain tissue morphology, MRI was performed on wild-type (WT) and α-Syn knock-out (KO) mice.

METHODS

Animal experiments were approved by and performed according to the Institutional Animal Care and Use Committee and conform to National Institutes of Health guidelines. Studies were conducted with male and femle C57BL6 mice (age 3–6 months) homozygous for targeted disruption of the gene encoding α-syntrophin (α-Syn –/–) (Ref. 2). C57BL6B WT littermates of the α-Syn –/– mice were used as controls.

MRI was performed at 7T on a Bruker Biospec 70/20 AS with BGA-12 400mT/m gradients and a 72mm volume resonator for transmit and an actively decoupled mouse brain quadrature surface coil for receive-only. Animals lay prone head first in the magnet, heated by a circulating water bed and body temperature and respiration maintained at 37±1C and 70±10/min respectively. Anaesthesia consisted of inhalation gas 67.5% N2/32.5% O2 + 1.5-3% isofluran. The MRI protocol consisted of tri-axial and sagittal 2D scout scans, localized shimming (FASTMAP), and sequences for quantitative estimation of T2 (T2-maps), diffusion (Trace-ADC with extended b-range) and perfusion (CBF by ASL). Key parameters for MRI:

2D T2-mapping (MSME-CPMG): TE=7.6ms (40 echoes), TR=2500ms, FOV=20x20mm, Matrix=128x96, Sl.thick=2mm (slice), NEX=2, AT=8min.

2D diffusion (DTI-EPIss) TE=35ms, TR=2000ms, FOV=20x20mm, Matrix=64x64, Sl.thick=2mm (1 slice), NEX=1, 3B0-images, 3 directions, 12 b-values at low (200-700), intermediate (1100-1700) and high (2400-3000) range, AT=1min18s.

2D perfusion (FAIR-EPIss) TE=20ms, TR=8000ms, FOV=20x20mm, Matrix=80x64, Sl.thick=1mm (1 slice), NEX=1, TIR-min=33ms, Number of TIR-values=50, TIR increment=150ms, Slice package margin for selective inversion=0.5mm, AT=13min20s for both selective and global inversion.

Image analysis were performed on the scanner console (PV4.0) and offline with MATLAB. Slices and ROIs were primarily selected to cover the visual cortex. Separate Trace ADCs were calculated for groups of 3 b-values at low, intermediate and high b-ranges (Figure 2). CBF was calculated from T_1-values [ms] obtained by fitting the formula: $S=S_0(1-(1+\alpha)\exp(-t/T_1))$ to the experimental data (selective and global separately). Cerebral blood flow was then calculated as $CBF=90*(60000/T_{1,SELECTIVE}-60000/T_{1,GLOBAL})$. Statistics were performed in Excel using oneway t-tests with unequal variances.

RESULTS AND DISCUSSION

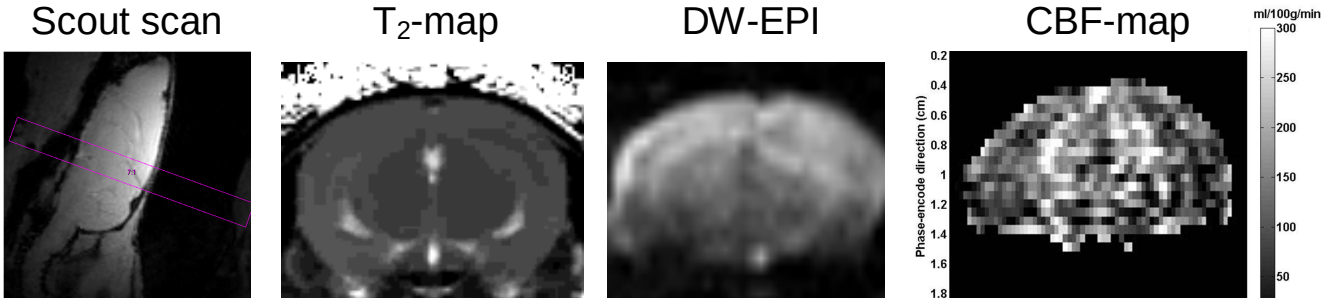

FIGURE 1. *7T brain MRI of α-syntrophin knock-out mice from the C57BL6 strain. Sagittal scout scan (A) with typical slice plan for T2-map (B), Diffusion-weighted MRI (C), and Perfusion-map (D).*

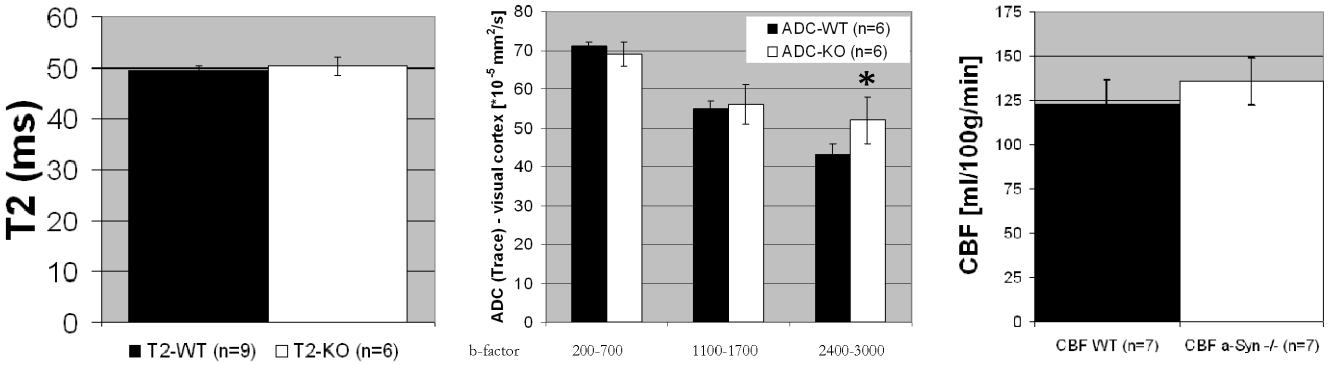

FIGURE 2. *Transversal relaxation time (T2), Apparent diffusion coefficients (Trace ADC) at increasing b-value ranges and Cerebral Blood Flow (CBF) in wild-type (WT) and α-syntrophin KO mice from the C57BL6 strain. All values are Mean±SEM (bars). * indicates significant difference (p=0.008).*

In the resting state, α-syntrophin knock-out (KO) mice did not present significant deviations in cortical T2-values or CBF (Figure 2) and the values found for these parameters lie in the ranges reported by other groups. The Arterial Spin Labeling method used here (FAIR-EPI) is in addition to the actual animal physiology sensitive to some MRI parameters such as the slice margin in selective inversion mode and need to be optimized further. Brain water diffusion (Trace ADC) was significantly increased in the α-syntrophin knock-out KO mice when measured with high b-values (2400-3000 s/mm^2). This finding raises important questions: Firstly, does AQP4 water channel transport in the brain contribute to or affect the MRI derived measure of diffusion (ADC)? Secondly, is the redistribution of the AQP4 proteins in the astrocyte membranes causing a transition of slowly diffusing spins into a more mobile state? The notion of swelling astrocyte end-feet in α-syntrophin knock-out mice (Ref.3) calls for additional MRI studies to further understand the nature of altered water balance in the brain in these animals, both in resting and activated states.

CONCLUSIONS

We have shown that MRI phenotyping of α-syntrophin KO mice in the resting state reveals subtle changes in apparent water diffusion in the cortical areas of the brain. These findings suggest a role for MRI in understanding the function of AQP4. In addition, the animal model used here may serve as a tool in deciphering of the signal attenuation versus b-value curve obtained by diffusion weighted MRI.

REFERENCES

1) Amiry-Moghaddam, M. etal. PNAS 100(23) Nov 11 (2003), pp. 13615-20.; 2) Adams, ME etal. J. Cell Biol 150 (2000), pp. 1385–1398.
3) Amiry-Moghaddam, M. etal. PNAS 100(4) Feb 18;: 2003: pp.2106-11.