*In vivo*9.4T 1H MRS for evaluation of brain metabolic changes in the Ts65Dn mouse model of Down syndrom

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Introduction:

Down Syndrom (DS) (human trisomy 21) is a chromosomal abnormality characterized by the presence of an additional copy of some genes on chrosome 21. This pathology is characterized by a set of behavioural, morphological and metabolic alterations. Ts65Dn model is the most widely studied mice model for DS [1]. This mouse is trisomic for the chromosome 16 which is homologous to human chromosome 21. Many studies have also been performed using NMR spectroscopy to characterize cerebral degenerative diseases in humans. It has been shown that a decrease (13%) of N-acetylaspartate is related to a process of cerebral degeneration in Alzheimer's disease in Down syndrome cases and in children mental retardation cases [2]. Another metabolite (myoinositol) is increased in adults DS [3]. Similarly, mass spectrometry studies confirmed an elevated level of myoinositol in the hippocampus, cerebellum and frontal cortex of Ts65Dn trisomic mice [4]. Therefore it is interesting to explore cerebral metabolism of Ts65Dn models. *In vivo* Magnetic Resonance Spectroscopy (MRS), which is known to be a non invasive method, can provide information on brain metabolism and neuronal function. The aim of our study was to quantify changes in brain metabolites concentrations for TS65Dn mice compared to control mice with 9.4T 1H MRS.

Material and methods:

10 mice were used in this study. They were divided into two groups: the first one included 5 Ts65Dn mice and the second one included 5 wild-type (2n) mice. MRS experiments were performed on a 9.4 T horizontal magnet (94/20 USR Bruker Biospec, Wissembourg, France) with a 35mm diameter birdcage coil. During the MR experiments, mice were placed in a custom-built cradle to immobilize their head. They were anesthetized during MR experiment with 1.5% isoflurane and a mixture O_2/N_2O (1:1) with an output of 0.7L / min. Respiratory motion was monitored during all the experiment using a air pillow. Mice body temperature was maintained constant with a warm water circulation.

A PRESS sequence (TR=4s, TE=15ms) with water suppression (VAPOR) and Outer Volume Suppression (OVS) was used to record 1H spectra in a 2*2*2mm voxel in the hippocampus and in the cerebellum. 256 scans were performed for a total acquisition duration of 17 min. Several metabolites (NAA, choline, myo inositol, glutamate, glutamine) were then quantified on the spectra with AMARES software [7],. Mann Whitney test were performed to compare metabolites concentration between 2n and TS65Dn mice.

Results:

The spectra were analyzed and quantified for hippocampus and cerebellum with AMARES method by JMRUI software [7]. The area under each metabolite peak (Lorentzian-Gaussian). was determined by integral calculation. It takes into account the amplitude, width at half height and the width at the foot of the peak. Thus the area calculated is directly proportional to the concentration of metabolite in the considered region which is expressed as relative quantity (NAA/Cr, Gln+Glu/Cr, Cho/Cr, Ins/Cr, Tau/Cr and Lac/Cr). In our study total Creatine concentration (CR+PCr) was considered as constant and we arbitrarily assigned a value of 1 at this concentration to express other metabolites concentrations as relative quantities.

Quantification of metabolites for the cerebellum and hippocampus are presented respectively on figures 1 and 2. For the cerebellum there was a significant decrease in myo-inositol (p=0.04), NAA (0.036) and glutamate+glutamine (Glx) (p=0.004) concentrations for Ts65Dn mice. No significant variations in NAA were observed between 2n and Ts65Dn mice.

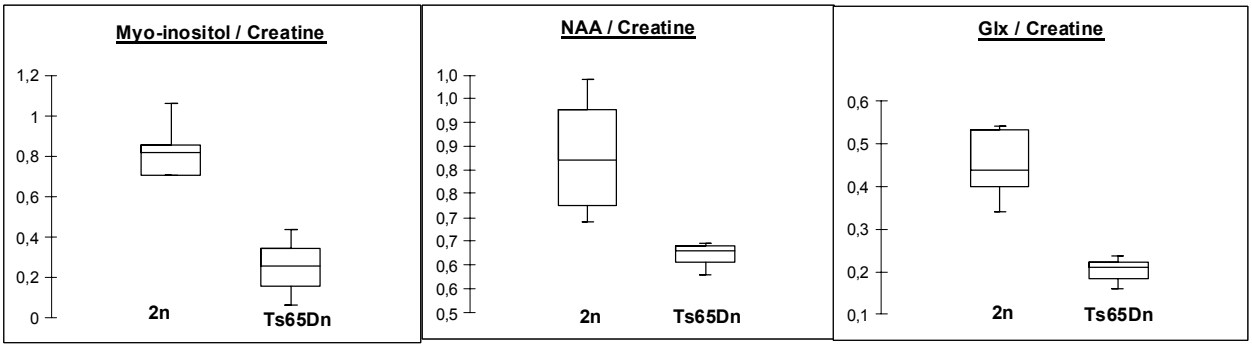

Fig.1 : Comparison of Myo-inositol, NAA and glutamate+glutamine pool in the cerebellum for 2n and Ts65Dn mice
(Graph vertical scales indicate relative concentration of metabolites relative to total créatine)

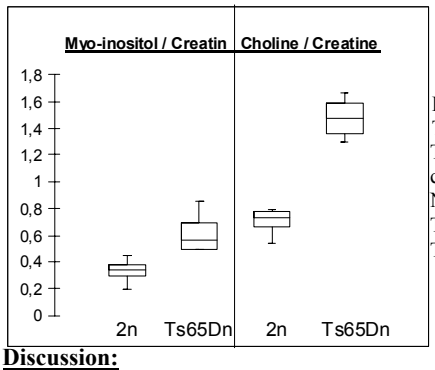

Fig.2: Comparison of Myo-inositol/total creatine, and choline/total creatine in the hippocampus for 2n and Ts65Dn mice
There was a significant increase in Myo-inositol (p= 0.031) and choline (p=0.025) concentrations for Ts65Dn mice.
No significant variation in NAA and glutamate+glutamine pool was observed between 2n and Ts65Dn mice.
There was a nonsignificant decrease of NAA (p=0.06) and glutamate +glutamine pool (p=0.06) for Ts65Dn mice.

Discussion:

Our 9.4T 1H MRS study is the first *in vivo* study performed on trisomic mice. It showed cerebral metabolism perturbations in hippocampus and cerebellum for Ts65Dn mice. In the cerebellum the decrease in myo-inositol may be related to a decrease in granular cells density. In the hippocampus the increase in myo-inositol may be related to the presence of SLC5A3 gene in three copies which codes for the Na+-myo inositol cotransporter [5]. The decrease in glutamate+glutamine pool may be related to neuronal loss or glial default [6]. In conclusion this present work underlines the feasibility of MRS and its usefulness to characterize brain metabolic changes during DS or more generally to characterize metabolic profile of different mice mouse strains or for different pathologies.

References:

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