White Matter Damage in Nijmegen Breakage Syndrome by Diffusion and T2 MRI

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Introduction

Nijmegen Breakage Syndrome (NBS) is a rare autosomal recessive disorder. Mutation in the NBS-1 gene was found to be the cause of this disorder. The NBS-1 gene produces nibrin, a protein believed to play a significant role in DNA damage response. NBS patients suffer from many of the symptoms of Ataxia-telangiectasia (variant V1) including immunodeficiency, chromosomal instability and lymphoreticular malignancies. The disorder itself causes brain microcephaly, that leads to severe developmental disorders and often pre-mature death of the subjects. NBS subjects are highly sensitive to invasive radiation thus CT, PET and IR are extremely harmful for these subjects. Consequently, MRI and US are the methods of choice for the diagnosis and follow-up of this disorder. In humans, MRI of NBS subjects shows significant increase in cerebro-spinal fluid volume, abnormal development of tissue contrast, microcephaly and solid tumors.

Recently, conditional inactivation of NBS1 in the central nervous system was generated in mice [NBS1-(CNS)-del]. In this work we have conducted an MRI study on conditional NBS1-(CNS)-del (knock-out, KO) and wild-type (WT) mice in order to characterize, for the first time, the *in-vivo* appearance and integrity of various brain structures in NBS1 deficient mice. Studying this model might shed light on the pathophysiological roles of this gene and its relation to neurodegenerative processes.

Methods

Nine KO and 9 WT mice underwent MRI in a 7T/30 spectrometer (Bruker). The two mice groups were scanned *in-vivo* at the age of 3 months. The MRI protocol included T1-weighted images (TR/TE=1000/14ms), multi-echo T2-weighted images (TR=3000, TE linearly incremented from 10ms to 120ms) and diffusion weighted echo planar images (DWI-EPI, TR/TE=3000/25ms, 4 EPI segments, Δ/δ=10/4.5 ms, b value of 1,000 s/mm^2). In all experiments the FOV was 20mm with matrix of 192x160 (for the T1 and T2 experiments) and 96x80 for the DWI-EPI. Eight slices of 1.2mm thickness and no gap were acquired both in axial and sagittal orientations. The total MRI scanning time was approximately 30 minutes.

After the MRI, the animals were sacrificed, the brain extracted and prepared for immunohistochemistry staining for Myelin Basic Protein (MBP), Sytox (cell nucleus staining) and GalC (mature oligodendrocytes staining)

Results

T2 weighted MRI revealed significant morphological and contrast changes between the KO and WT groups. First, the brains of the CNS Nbs1-del mice were significantly smaller than those of the WT with emphasized mal-development of the cerebellum. In addition, the typical hypo-intense white matter signal in the corpus callosum ,internal-capsule and cerebellar folia almost disappeared. Region of interest analysis of the T2 maps revealed significant T2 changes in the areas of white matter (corpus callosum, internal capsule, mid brain and optic nerve) with minor changes if all in gray matter (Figure 1). This pattern of white matter damage repeated in the DTI analysis where it was found that FA values are significantly reduced in the abovementioned areas (Figure 2). The FA reduction was mainly due to increase in the radial diffusivity.

Biochemical analysis revealed that NBS1-(CNS)-del mice show low and dispersed staining for MBP and GalC (an oligodendrocyte marker). The oligodendrocyte density in KO mice is significantly lower compared to WT and with a pre-mature appearance of those cells in the KO brains (see sections of the cerebellum in Figure 3).

Discussion and Conclusions

In this study we showed that diffusion and T2 analysis can be used for characterization of tissue damage in transgenic mice, specifically in the animal model of NBS. The combination of T2 and DTI points to massive damage to white matter areas in this animal model. This indicates high vulnerability of the white matter system for knock out of the NBS gene which is essential component of the DNA damage response. Combined with histological analysis it seems that the mal-development of the white matter is rooted in immature or defective development of the oligodendrocytes that support the axonal network. Damage to white matter is not probably the only change that occurs when such a key gene is knocked out but it seems that the white matter damage plays a significant role if not the leading role in the degeneration of NBS1-(CNS)-del mice brains.

Figure 1

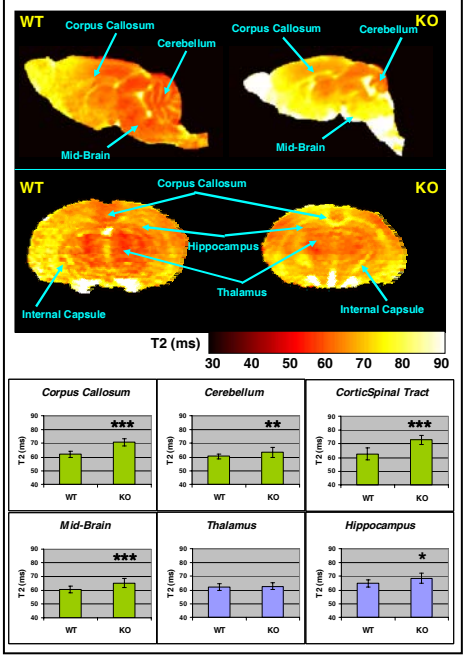
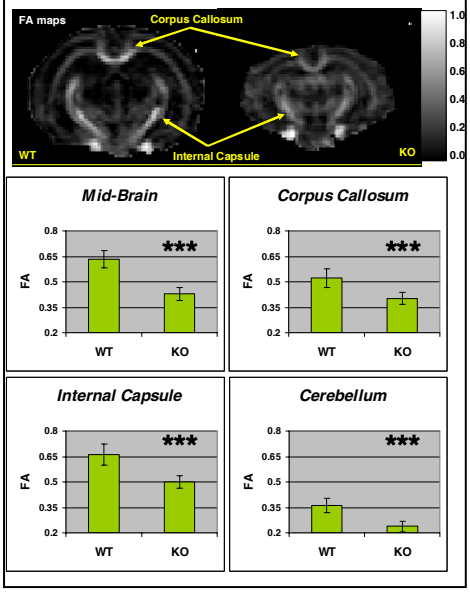


Figure 2



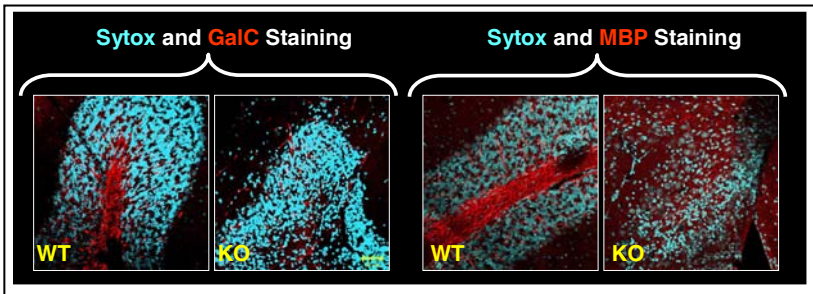


Figure 3