

Brain N-acetylaspartate is Increased in Mice with Hypomyelination

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INTRODUCTION

N-acetylaspartate (NAA) is a compound found predominantly in neurons and their processes, and is generally considered to be an important marker of viable, functioning neurons and axons. Many neurodegenerative disorders, therefore, exhibit a decrease of total NAA (tNAA; NAA at 2.01 ppm and N-acetylaspartylglutamate [NAAG] at 2.04 ppm, which are difficult to distinguish on clinical ¹H-MRS with a 1.5 or 3.0 tesla magnet). Previous studies have shown the elevation of tNAA on ¹H-MRS with 1.5 tesla magnet in Pelizaeus-Merbacher disease (PMD), a representative hypomyelination disease. In order to evaluate a hypothesis that hypomyelinating process may affect NAA and NAAG biochemical pathways, we examined *myelin synthesis-deficient* (*msd*) mouse brain, a model of PMD with a 7.0 tesla magnet and separately measured NAA and NAAG of the brains of these animals by high performance liquid chromatography (HPLC).

MATERIALS AND METHODS

The experimental animals were *msd* mice with a spontaneous mutation (A242V) in the *plp1* gene (maintained in B6C3 background; n=9, weight=6.7±1.4g), and wild-type littermate mice (n=10, weight=11.3±1.2g) at P21.

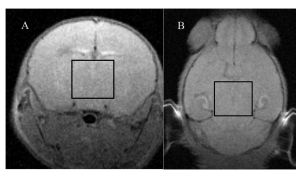
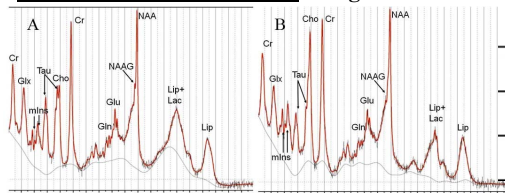


Fig. 1 All MRI experiments were performed on a 7.0 tesla MRI scanner (Magnet: Kobelco and JASTEC Japan; Console: Bruker Biospin, Germany) with a volume coil for transmission (Bruker) and a 2-ch phased array coil for reception (Rapid Biomedical, Germany). Single voxel ¹H-MRS of the thalamus (Fig. 1) was performed with a VOI of 3x3x3mm. Outer volume suppression (OVS) combined with a point-resolved spectroscopy (PRESS) sequence was used for signal acquisition (number of repetitions=256; TR=2500ms; TE=20ms; spectral bandwidth=4 kHz; number of data points=2048; scan time=10 minutes 42 seconds). ¹H-MRS was quantitatively analyzed using the LCModel. NAA and NAAG in the brain were independently measured by high performance liquid chromatography (HPLC). Immunohistochemical analysis using anti-Mbp (marker for mature myelin sheath), Gfap (marker for astrocytes) antibodies were performed.

RESULTS and DISCUSSION

Fig. 2

Table

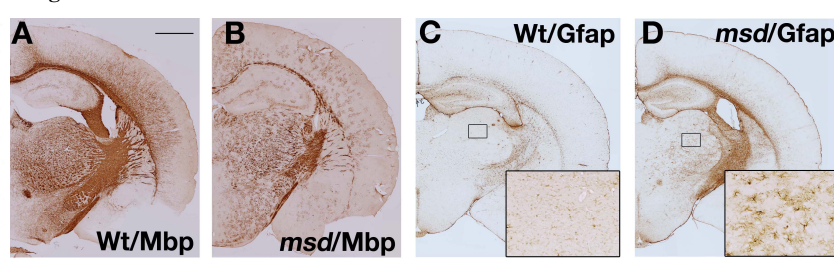
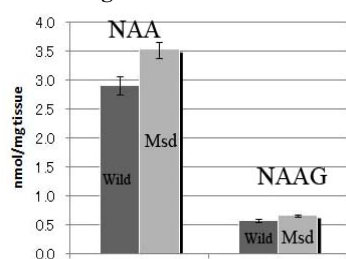


	tNAA	Cr	Cho	mIns	Gln	Glu	Tau
Msd	7.11±0.31	6.17±0.31	1.11±0.06	4.13±0.42	4.23±0.47	7.44±0.41	7.26±0.88
Wild	5.45±0.51	5.73±0.15	1.45±0.10	4.29±0.51	3.89±0.55	6.43±0.32	6.42±0.49
p value	<0.001	<0.001	<0.001	NS	<0.01	<0.001	<0.01

¹H-MRS in *msd* mice (Fig. 2-A, Table) revealed increased tNAA, creatine (Cr), glutamine (Gln), glutamate (Glu), and taurine (Tau), and decreased choline (Cho), compared with wild-type mice (Fig. 2-B). NAA and NAAG could not be separately analyzed by ¹H-MRS.

Fig. 3

Fig. 4



HPLC analysis revealed increases of both NAA and NAAG in the *msd* brains (Fig. 3). Immunostaining of Mbp in *msd* mice showed sparse and weak staining in the

white matter, thalamus and cortex in comparison with in the wild-type (Fig. 4-A, B). Gfap immunostaining in the *msd* brain showed dense and strong staining and an increase of positive cells compared with in the wild type (Fig. 4-C, D). These indicated hypomyelination and astrogliosis in the *msd* brain at P21.

NAA is either released from the neuron or transported to oligodendrocytes, where it is catabolized by aspartoacylase (ASP) into acetate and aspartate. NAA is also the precursor for the synthesis of NAAG in neurons. In *msd*, mutant *plp1* proteins are abnormally folded and accumulated in the endoplasmic reticulum, resulting in the activation of unfolded protein response that finally leads to oligodendrocyte apoptotic cell death before normal myelination occurs. The absence or dysfunction of mature myelinating oligodendrocytes, where NAA is catabolized by ASP, may either disable the neuron-to-oligodendrocyte NAA transport or affect NAA catabolism in oligodendrocytes. In either way, NAA would be accumulated in neurons. Because elevated NAA concentration increases NAAG biosynthesis, NAAG may be secondarily increased. The reduction of Cho may be explained by the severe retardation of myelination and the diminished number of oligodendrocytes in *msd* mice brain (Fig. 4). The elevated Cr and Tau observed on ¹H-MRS may result from the increased number of astrocytes which are rich in Cr and Tau.

CONCLUSION

Hypomyelination could affect the NAA and NAAG biochemical pathways leading to increase both of them. Increased tNAA with decreased Cho detectable on ¹H-MRS may be an important marker for hypomyelinating disorders, which can be distinguished from more common neurological disorders that have decreased tNAA.