Ultra High Field Magnetic Resonance Microimaging in Zebrafish Model of Cystic Leukoencephalopathy

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Introduction

Zebrafish is increasingly used as model organism for understanding brain diseases including neurodegenerative disorders. Cystic leukoencephalopathy is a demyelinating disease that manifest with psychomotor impairment, micro- or normocephaly, spasticity and epilepsy [1, 2]. Recent studies have shown that RNASET2 deficiency in man results in leukoencepgalopathy that is characterized by cortical cysts and multifocal white matter lesions [3]. Based on this very recently the first zebrafish model for Cystic Leukoencephalopathy has been developed in which RNASET2 gene has been mutated [4]. In this study we applied high resolution μMRI to monitor if this zebrafish model has similar characteristics in terms of cortical cysts and white matter lesions as found in human patients with cystic Leukoencephalopathy. Our results show that RNASET2-deficient zebrafish has similar diagnostic pattern as found in RNASET2 deficient human and suggest that RNASET2-deficient zebrafish could serve as an excellent animal model for Cystic Leukoencephalopathy. Furthermore this study demonstrates that μMRI can be successfully used to visualize neurodegeneration in zebrafish.

Methods

Adult wild-type (WT) zebrafish (n=5) and RNASET2 deficient zebrafish (n=5) were euthanized and were fixed in 4% buffered pfa for 2 days and subsequently embedded in Fomblin. All μMRI measurements were conducted on a 400 MHz (9.4T) vertical bore system [5], using a transmit/receive birdcage radiofrequency coil with an inner diameter of 10 mm and a 1 Tm^{-1}gradient insert (Bruker Analytic, Germany). For high resolution, T_2-weighted MR images were acquired by a rapid acquisition with relaxation enhancement (RARE) sequence [echo time (TE) = 10.567 ms (22.45 ms effective), repetition time (TR) = 5s, averages = 64, echo train length = 4]. The field of view was 1.2 x 1.2 cm^2, with image matrix of 256 x 256 yielding an effective in plane resolution of approximately 47 μm. For T_2 relaxation time measurement, a multislice multi echo (MSME) sequence with was used. Imaging parameters were: FOV 2.0 x 2.0 cm^2, matrix size 256 x 256, number of averages 2, number of slices 6 with slice thickness of 0.5 mm, number of echos 12 with TE of 8.5, 17.0, 25.5, 34.0, 42.5, 51.0, 59.5, 68.0, 76.5, 85.0, 93.5, 102.0 ms and a TR of 1.5 s. T_2 was calculated from the plot of TE versus T2 contrast (magnetization present in x–y plane, Mxy) by applying the equation $M_{xy}(t)=M_{xy_{max}} e^{-1/T2}$. Immunohistology on paraffin-embadded tissue section (5μm) was performed using anti-amyloid precursor protein as described elsewhere [4].

Results and Discussion

Zebrafish with mutation in RNASET2 gene showed frequent white matter anomalies which were scattered throughout the brain. Fig. 1 show T2-weighted MR images of the brain of WT and *RNASET2* mutant zebrafish. The sagittal images indicate the presence of lesion (white arrow) in telencephalon of mutant zebrafish (Fig. 1b) which was not seen in WT (Fig. 1a). The coronal images in Fig. 2 showed dilated ventricles and the presence of lesions adjacent to the ventricles in *RNASET2* mutant zebrafish. The lesions adjacent to ventricles have similar T2 relaxation time as that of ventricles suggesting that these lesions may be filled with solution similar to cerebral spinal fluid (Fig. 3). Immunohistochemical examination of the brain of the same fish as shown in Fig. 1a and b show that axonal disruption occurs around the ventricles in mutant fish (Fig. 4). In conclusion, our results suggest that zebrafish model of Cystic Leukoencephalopathy develops similar lesions as found in human patients and show that μMRI can be successfully used to visualize neurodegeneration in zebrafish.

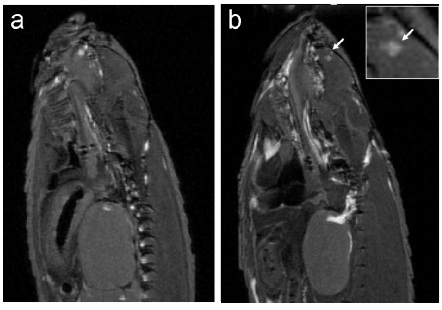

Fig. 1: *T2-weighted MR images of the brain of (a) control and (b) mutant zebrafish containing mutation in RNASET2 gene. The sagittal slices were obtained with an in-plane resolution of 39 μm. The magnified sub-sampled area of (b) is shown in the image on right indicating the presence of lesion (white arrow) in mutant zebrafish in telencephalon which was not seen in control fish.*

References: [1] Henneke M. *et al. Neurology* 2005;64;141. [2] Faria E *et al*. Arq Neuropsiquiatr 2008;66:261. [3] Henneke, M. *et al.* Nature Genetics 2009; 41:773. [4] Haud N. *et al.* PNAS 2010 (accepted). [5] Kabli S et al. Zebrafish, 2010; 7:143-148.

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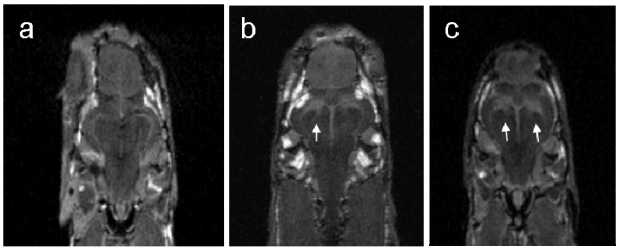
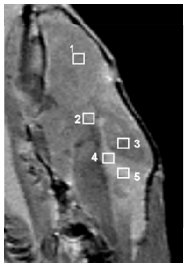


Fig. 2: *T2-weighted MR images of the brain of a control (a) and two mutant zebrafish containing mutation in RNASET2 gene (b, c). The horizontal slices were obtained with an in-plane resolution of 39 μm. Arrows indicate the presence of lesions adjacent to the ventricles and were of similar intensity as that of cerebrospinal fluid present in ventricles in both the mutant zebrafish.*



Area	T_2 relaxation
1	28.3±1.02
2	31.09±1.31
3	25.01±1.26
4	32.14±0.99
5	33.7±1.04

Fig. 3: *T2 relaxation time measurement of the brain of RNAseT2 mutant zebrafish. Areas selected for T2 relaxation time measurements within healthy brain region (1, 3), in ventricles (4) and in lesion outside (2) or adjacent to ventricle (5).*

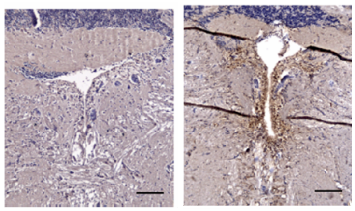


Fig. 4 *Immunohitochemical examination of control (a) and RNASET2 mutant zebrafish (b). Immunohistology was carried out on the same zebrafish as shown in Fig 1a and b. Axonal disruptions around ventricles can be clearly seen in mutant fish*