Screening Mutagenic Mice with Ventricular Mutation

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Synopsis
To screen mice with ventricular mutation, a normal mouse ventricular volume library (NMVVL) of different age groups and ventricular compartments is established from the high resolution 3-D T2-weighted MRI images. The ventricle of a mutagenic mouse is then compared with NMVVL for a primary screening test.

Introduction
Genetic disorders study has become one of the most important topics in medical science **[1]**. Phenotype-driven approach to induce large scale of mutant mouse by using chemical mutagen, N-ethyl-N-nitrosourea (ENU) is a recent development to mimic and uncover the mechanisms of gene mutation **[2]**. To screen mice with ENU-induced brain disorders, one needs a none-invasive method for detection and distinction, which makes MRI one of the best candidates to use. Because the abnormal enlargement of ventricles have been found on mice with many brain disorders, such as schizophrenia, Alzheimer's disease, and benign intra-cranial hypertension etc.**[3]**, the comparison of ventricular volumes could be used as the criteria for ENU-induced brain disorder mice. It is, therefore, the goal of this study to establish a set of normal mice MRI data library focused on ventricle, and demonstrate its usability for screening by comparing it with a set of brain disorder mice MRI data that is induced by ENU.

Material & Methods
Test Subjects & MRI Acquisition: 24 male C57BL/6J mice were used for the control population of this study. The mice were divided into four subgroups. Group 1 included six 3-4-week-old mice (11.08±2.35g). Six 5-6-week-old mice, weighing 18.08±2.75g, constituted Group 2. Group 3 contained six 15-21-week-old, 26.30±1.06g (μ±σ) mice. Group 4 was made up of six mice older than 28 weeks (27.63±1.23g). The comparison group was comprised of an ENU-induced mutagenic mouse (male, 37 weeks old, and 29.35g).
The MRI scans were performed on a Bruker, Pharmascan (7 tesla) scanner. Sagittal T2-weighted image (TR = 2000ms, TE_{eff} = 88.3ms, FOV = 3 cm; Matrix = 256×128) was acquired and used for the location and length determination of the brain. A T2 weighted 3-D RARE scans was acquired axially (TR = 4000ms, TE_{eff} = 80ms, voxel size = 2.86×10^{-6} cm, Matrix = 256×128×64).

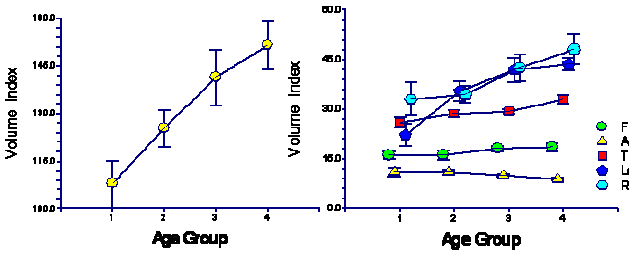

Fig. 1 Total Ventricle

Fig. 2 Individual Part of the Ventricle

Normal Mouse Ventricle Volume Library: To eliminate human errors, two imaging analysts, blind to the test subjects, were responsible for manual depicting ROIs of ventricle. ROIs of ventricle are delineated carefully into the right lateral ventricle, the left lateral ventricle, the 3rd ventricle, aqueduct, and the 4th ventricle. The voxels assigned to these individual parts of ventricle were summed and multiplied by the voxel size
to obtain their volumes respectively, and the total ventricular volume is the sum of all these separate parts, which makes the bases of the NMVVL. All images were processed using manual trace tool and edge editing function provided by ANALYZE (Biomedical Imaging Resource, Mayo Foundation, Rochester, Minn).

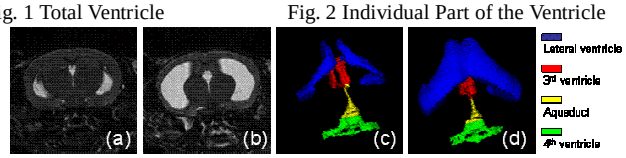

Fig. 3 Representation T2-weighting images of (a) control mouse (29.15g) (b) abnormal mouse (29.35g) and 3-D reconstruction images of (c) control mouse (29.15g) (d) abnormal mouse (29.35g)

The obtained NMVVL is divided into four age groups to account for the size deviation. In addition, a volume index is introduced to correct individual volume deviation introduced by each mice of the same group. The volume index is defined to be the ratio of the ventricular volume and the brain length. The brain length is defined by the length from the boundary between the olfactory bulb and forebrain to the bottom of the cerebellum in the mid-sagittal slice of the brain. The mean and standard deviation of all the measurements are subjected to paired t tests for reliability examination, and the analysis of variance (ANOVA) for revealing the relation between each different age group and different ventricular parts ($p<0.05$ is considered as having significant difference).

Results
In the reliability examination, there are no statistically significant differences, based on paired t tests, between any volume measurements obtained by each analyst. Table 1 shows NMVVL data and an ENU-induced mutagenic mouse data categorized into groups of the average of the brain lengths and ventricular volume indexes. The relation between the total ventricles and the age groups is showed in Fig. 1, and individual parts of the ventricle in Fig. 2. It clearly shows that the ventricular volume index, especially the lateral ventricle (Fig. 2), increases as the age of the individual increases (Fig. 1). Fig. 3 shows the comparison of the ENU-induced mutagenic mouse and one of the normal mice in Group 4 taken from NMVVL.

Table 1. NMVVL data of different age groups vs. ENU-induced mutagenic mouse data

	Age (weeks)	Brain Length (mm)	Total Ventricle (volume Index unit × 100)	4th Ventricle (volume Index unit × 100)	Aqueduct (volume Index unit ×100)	3rd Ventricle (volume Index unit × 100)	Left Lateral Ventricle (volume Index unit × 100)	Right Lateral Ventricle (volume Index unit × 100)
Group 1	3-4	11.53±0.31	108.01±19.48	16.07±3.17	10.86±3.08	25.92±3.89	22.14±8.34	33.03±12.66
Group 2	5-6	11.90±0.20	125.28±12.37	16.07±2.80	10.91±1.60	28.53±2.47	35.39±6.59	34.37±5.69
Group 3	15-21	12.42±0.25	141.40±22.16	18.04±2.20	9.93±0.80	29.21±2.16	41.75±8.82	42.47±10.52
Group 4	>28	12.40±0.24	151.63±20.09	18.45±3.04	8.79±1.01	32.84±3.39	43.50±5.00	48.05±11.69
ENU mouse	37	12.80±0.05	697.10±7.58	18.88±0.10	7.45±0.20	42.50±1.11	322.61±1.47	305.67±5.61

Discussion
In Fig. 1, the total ventricular volume increases significantly with age increasing in each age group. However, Fig. 2 shows that the changes in 4th ventricle and aqueduct are much less than in 3rd and lateral ventricle. The ANOVA test has also shown significant differences between the age groups of total ventricular volume index ($p<0.05$), the 3rd and lateral ventricle, but not in 4th ventricle and aqueduct groups. This might indicate that the growing of 3rd and lateral ventricle continues, long after the 4th ventricle and aqueduct have reached their mature stage. These differences between age groups and each parts of ventricle should all be taken into account when the normal library is used to screen the mice with abnormal ventricle. Interestingly, in Fig. 3, the difference between lateral ventricles of ENU-induced mutagenic mouse and the normal mouse is also more significant than the rest of the ventricle (c.a. 7 times in size). The same phenomena can be observed in the 3rd ventricle but with a lesser difference (c.a. 1.3 times in size). Although the detail mechanism of the ENU-induced mutation is still unknown at this stage, as a proof of principle, this study shows the feasibility of ventricular volume detection to screen ENU induced mutant mice. The investigation into more efficient and appropriate auto-segmentation method will be considered in the future direction.

References
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