Normal Brain Aging and its Risk Factors –Analysis of Brain MRI Database of Healthy Japanese Subjects

Hiroshi Fukuda*[1)], Yasuyuki Taki[1)], Shigeo Kinomura[1)], Kazunori Sato[1)], Ryoi Goto[1)], and Ryuta Kawashima[2)]

* Professor

1) Department of Nuclear Medicine and Radiology, Institute of Development, Aging and Cancer

2) Department of Neuroimaging, Institute of Development, Aging and Cancer

E-mail: hiro@idac.tohoku.ac.jp

Abstract

We collected brain MRIs of 2,000 healthy Japanese and constructed an MRI database together with their characteristics such as age, sex, blood pressure, present and past disease history and cognitive functions. Volumetric analysis revealed that gray matter volume linearly decreased with time, while white matter volume remained unchanged during aging. Based on the regression line for gray matter loss, we defined a standard brain for each age group and sex. Correlation analyses using voxel-based morphometry (VBM) between gray matter volume and risk factors revealed that there were negative correlations between gray matter volume and hypertension, lifetime alcohol intake, obesity (BMI), mental status (subthreshold depression). Longitudinal study with 8 years interval revealed that there are sex difference in speed and pattern of gray matter loss with age.

To clarify clinical significance of ischemic changes in the white matter, we developed an algorism for automatic detection of the ischemic changes. Using this system, we made probabilistic map of the lesion for age of 40th, 50th, .60th and 70th. The lesion map revealed that ischemic lesion localized mainly around the ventricle and total lesion volume increased with increasing of age.

1. Introduction

To clarify the structure and functional mechanisms of human brain is one of the most important targets for medical sciences in 21st century. By using sophisticated techniques in rapidly growing molecular biology, we can clarify the molecular mechanisms for neurotransmission and neural mechanisms. These techniques are advantageous for clarifying the basic principles of neural function in mice and rat, however, these can not be applied to living human brain. The other critical problems is that mouse and rat have very small frontal lobe and results from these research can not be directly deduced to human brains that has very large frontal lobe.

Recent development of neuroimaging technique, such as MRI, PET (positron emission tomography), SPECT (single photon emission computed tomography) and optical imaging using NIRS (near infrared spectroscopy) enables us to visualize structure and function of the human brain. The importance of human neuroimaging database was recognized greatly and described in scientific journal (Science 297, 2001, J Neuroscience, 22, 2002). The most important purpose and needs for database are electric data sharing in neuroscience community as well as the construction of more sophisticated and complete model of brain structure and function. This model of normal brain structure and function can be used as the references not only for neuroimaging research but also for computer based objective diagnosis of the brain diseases.

There are many groups aiming neuroimage database development. Among them, ICBM (International Consortium for Brain Mapping), NeuroGenerator and fMRI Data Center are well known and powerful multi-centered groups. ICBM was founded in 1993 with a grant from the NIHM and the primary goal of the ICBM project is the continuing development of a probabilistic reference system for the human brain. This consortium is composed of four core research sites, UCLA, Montreal Neurological Institute (MNI), University of Texas at San Antonio, and the Institute of Medicine, Juelich/Heinrich Heine University - Germany. In addition, data acquisition sites in Asia (Sendai, Japan) and Europe (France, Finland, Netherlands) contribute to this international consortium. We are one of the members of ICBM and only one group in Japan and Asia involving the human brain database project. In this report, the activities of MNI and Sendai group will be introduced.

Most remarkable recently developed method for brain image analysis is a voxel-based morphometry (VBM). It includes anatomical standardization of the brain to a standard brain, brain tissue classification, and finally voxel-based statistical analysis based on general linear model. This technique enables us to extract brain regions which show correlations between tissue volume and dependent variables, such as age, sex and subject's characteristics. We can analyze not only age-related normal changes but also diseased brain, such as dementia and schizophrenia. It has been believed that functional imaging precede structural imaging to detect early pathological findings of the diseases. However, recent development of high resolution structural imaging and sophisticated analytical technique enable us to detect the brain disease at very early stage.

We have collected 2,000 brain MRI of healthy Japanese and constructed an MRI database together with their characteristics such as age, sex, blood pressure, present and past disease history and cognitive functions (Aoba-1 database)[1]. This is a largest database in Japan and one of the largest one in the world. Figure 1 shows number of subjects for each age and sex group. The age of the subjects distributed from 20th to 70th and each group has more than 80 subjects. Consequently, the database is very good material for brain aging analysis. In this report, we analyzed age-related structural changes of the Japanese healthy brain and their risk factors.

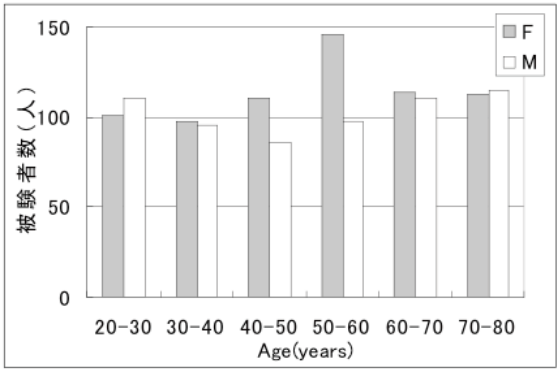

Fig. 1. Number of subjects for each age and sex group in brain MRI database

2. Methods

2.1. Subjects

Table 1 shows description of subjects. In this study, we analyzed 860 men and 840 women. Body mass index, blood pressure, percentage in smoking and drinking habit, diabetes mellitus, hypercholesterolemia and ischemic heart diseases of the subjects were described.

Table 1. Description of the subjects

	Men (n=860)	Women (n=840)
Age	44.5±17.8	47.6±15.1
Body mass index	23.4 ± 3.2	22.4 ± 3.1
Systolic blood pressure (mmHg)	129.5±16.5	125.7±18.2
Diastolic blood pressure (mmHg)	78.6±11.2	75.1 ± 11.9
Smoking habit (%)	62	17.1
Drinking habit (%)	91	65.4
Hypertension (%)	15.4	11.3
Diabetes mellitus (%)	4.3	2.1
Hypercholesterolemia (%)	7.2	12.1
Ischemic heart disease (%)	2.3	1.3

2.2. Anatomical standardization and tissue segmentation

First step of the image processing is anatomical standardization of the brain. All the brains with different size and shape were transferred into a standard stereotactic space (Talairach space) and their size and shape were transformed into a standard template brain using liner and non-linear parameters. After the process a given coordinate represents a specific anatomical structure in a specific region. Then, we can make voxel-based analysis without using region of interest (ROI) technique. This enables us to analyze a large number of images automatically in short time. Next step is brain tissue segmentation. A brain images were segmented into gray matter, white matter, cerebrospinal fluid space (CSF) and outer brain space depending on the differences of signal intensities on T1 weighted MRI of each tissue (Fig. 2). The voxel values of each segmented image consisted f 256 grade signal intensities according to their tissue probability. The volumes of each tissue segment were calculated by summing up a value which was calculated by the voxel volume multiplied by each voxel value divided by 255 in each voxel.

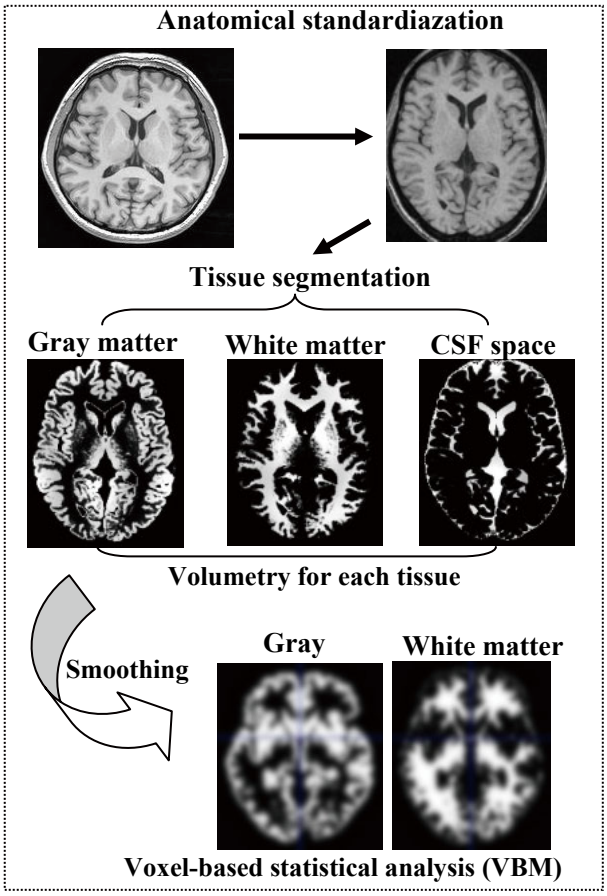

Fig. 2. Anatomical standardization and tissue segmentation of the brain MRI

2.3. Statistical analyses using VBM

The standardized and segmented gray matter images were smoothed by convoluting a 12-mm-FWHM isotropic Gaussian kernel. Then, the smoothed gray matter images were statistically analyzed by voxel based morphometry (VBM) technique using SPM2

package (Fig. 2). VBM was performed to investigate correlation between regional gray matter volume and attributes to the subject, such as cerebro-vascular risk factors, scores of cognitive and memory functions, and mental status. VBM enables the global analysis of brain structures without a priori identification of the region of interest. This approach is not biased toward any one brain region and permits the identification of unsuspected potential brain structural abnormalities. Simple or multiple regression analysis was performed using SPM2. These attributes were used as dependent variables, and regional gray matter volume as an independent variable. We set the significance level at $P<0.05$ for multiple comparison. The method is a voxel based t-statistics extended to the three dimensional space based on the general linear model. Simple or multiple regression analysis and group comparison using t-test were performed.

3. Results and Discussion

3.1. Brain volume change during aging

Figure 3 shows brain volume change during aging in each tissue segment. Gray matter volume linearly decreased with age in men and women. The slope of the regression line was significantly steeper in man than in women. On the hand white matter volume remained unchanged during aging, although variation of the data was large. The CSF volume increased with age [2]. This is a first report describing age-related change of the brain in healthy Japanese. Goods reported age-related volume change of the European brain [3]. Their results are consistent with ours.

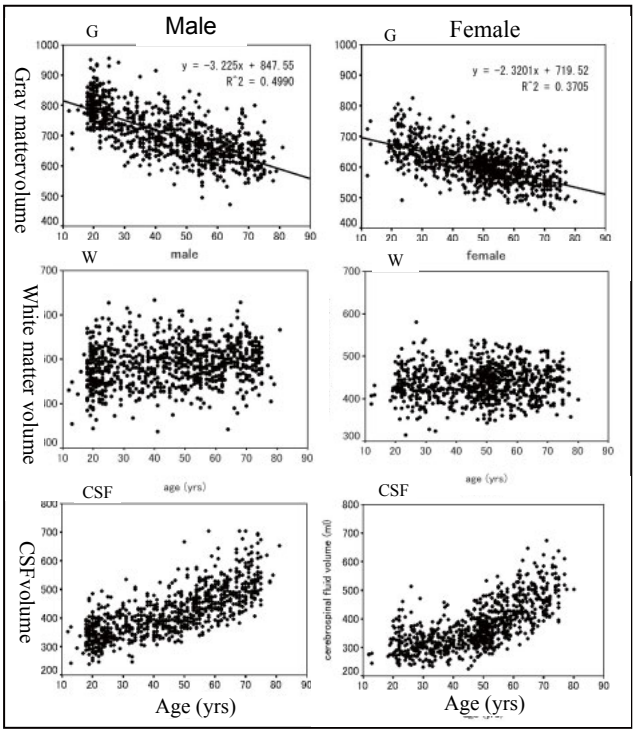

Fig. 3. Age-related volume change of the human brain. G: gray matter, W: white matter, CSF: cerebrospinal fluid space.

In order to normalize head size of the subjects, we calculated volume ratio of a given tissue segment to whole brain volume. The result in men was shown in Fig. 4. Gray matter ratio, which is the gray matter volume divided by whole brain volume, linearly decreased with age. Then, there was no statistical difference in the slope of the regression line for men and women. White matter ratio remained unchanged during aging, as was shown by white matter volume. CSF volume ratio increased with age [2](Fig. 4).

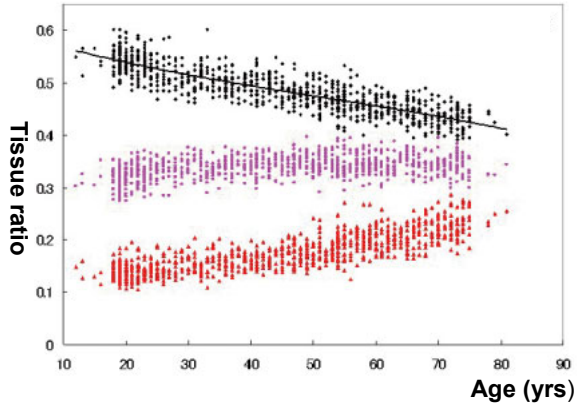

Fig. 4. Age-related volume change of the human brain Black: gray matter, pink: white matter, orange: CSF space.

3.2. Normally aged brain for a given age

Gray matter ratio decreased linearly with age. Therefore, a brain with mean gray matter ratio can be a representative brain for the age group. We calculated regression line for the gray matter ratio change with age (Fig. 5). We defined "a normally aged brain for his/her age", which is a brain with gray matter ratio on the regression line. This is a first objective criterion for normally aged brain. We determined the normal range of the gray matter ratio as the value within ±2SD from the mean value on the regression line.

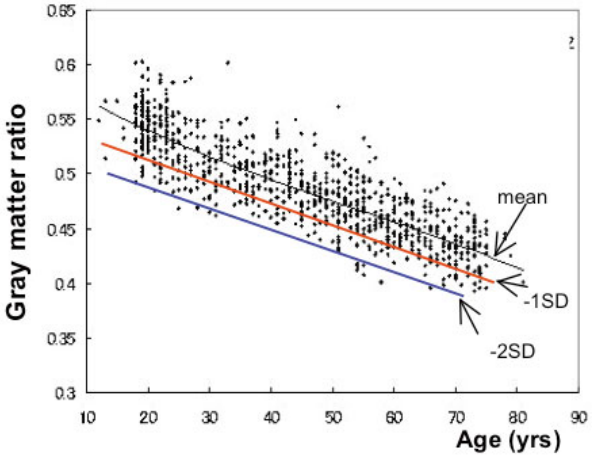

Fig. 5. Regression line for gray matter ratio change in men. Red and blue lines indicate minus 1SD and 2SD from the regression line.

Figure 6 showed normally aged brains for age of 40th, 50th, 60th and 70th, selected by this definition. The gray matter ratios for each age group were 0.442, 0.424, 0.406, and 0.385, respectively. The ventricles and sulci, as well as the extra brain space enlarged with increasing of age. These standard images can be reference brain images which will be useful for the objective diagnosis of brain atrophy.

Upper brain images on Fig. 7 showed a normally aged brain of 70 yrs old man determined by these criteria. The lower images represent atrophic brain for his age (70 years old), of which gray matter ratio exceeded -2SD limits. Enlargement of ventricle and sulci was observed compared to the normally aged brain.

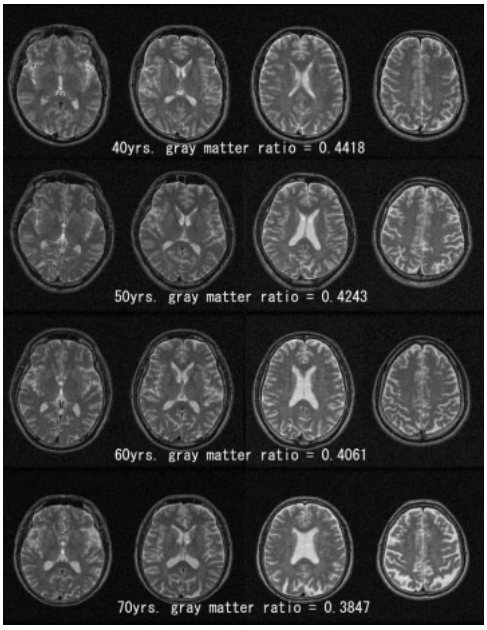

Fig. 6. Normally aged brain MRIs for age 20th to 70th (T2 weighted images)

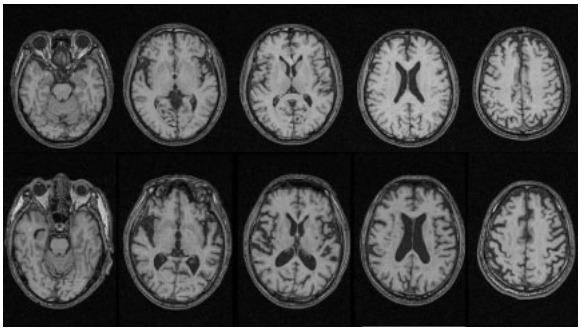

Fig. 7. T1 weighted brain MRIs of 70 year-old male
Upper column: normally aged brain images for age of 70.
Lower column: atrophic brain images for age of 70.

3.3. Longitudinal analysis of brain volume change during aging

Brain volume change described above are obtained from cross sectional analysis and do not represent age related change in strict meaning. Therefore, we performed a longitudinal study in the same subjects between 8 years interval for MRI study. The results indicated that gray matter ratio for men decreased linearly with age as was in our previous cross sectional analysis. While those for women decreased slowly than that for men until the age of 50 and then went down as the similar slope for men (data are not shown). White matter volume increased until age of 40 and went down thereafter in either men or women, although variation of the data was large.

3.4. Risk factors for brain volume decrease

3.4.1. Hypertension

We analyzed correlation between regional gray matter volume and subject's characteristics using VBM technique. We found that total gray matter volume negatively correlated with systolic blood pressure [2]. Figure 8 represents the brain regions that showed negative correlation between gray matter volume and systolic blood pressure. These regions mainly distributed watershed regions between major cerebral arteries, although some regions do not belong to the watershed regions.

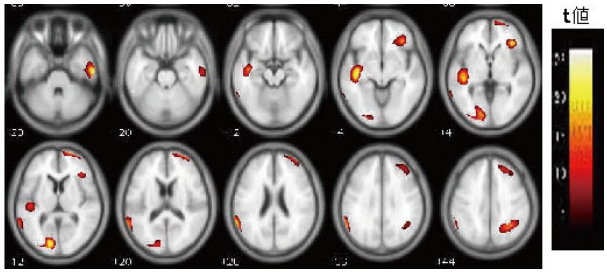

Fig. 8. Brain regions that showed negative correlation between gray matter volume and systolic blood pressure

3.4.2. Alcohol drinking

We also tested the correlation between gray matter ratio and life time alcohol intake. From the database, we selected 405 male subjects who drink alcohol less than once a week, but are not alcohol dependent. There was a strong negative correlation between the log transformed lifetime alcohol intake and the gray matter ratio. Figure 9 shows the gray matter regions that had a significant negative correlation between the lifetime alcohol intake and the regional gray matter volume. The gray matter volume of the bilateral middle frontal gyri showed a significant negative correlation with the log transformed lifetime alcohol intake [4]. Our results indicate that even non-alcohol-dependent subjects show brain volume reduction, just like as alcohol dependent subjects.

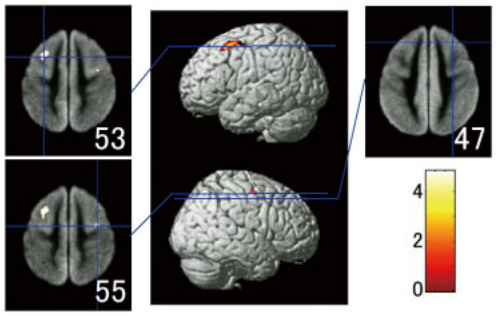

Fig. 9. Brain regions that showed negative correction between gray matter volume and life time alcohol intake

3.4.3. Obesity

We tested correlation between gray matter ratio and obesity. As an indicator for obesity, we used body mass index (BMI), which is a ratio of body weight (kg) to height $(m)^2$. Figure 10 shows histograms of BMI in men and women. The values distributed normally and mean values were 22 and 21 for men and women, respectively. Volumetric analysis revealed that there are significant negative correlation between BMI and the gray matter ratio, which represents the percentage of gray matter volume in the intracranial volume, in men ($p < 0.001$, adjusting for age, systolic blood pressure, and lifetime alcohol intake). On the other hand, we could not find any correlation in women.

In men, the regional gray matter volume of the bilateral medial aspects of the temporal lobe, bilateral anterior lobes of the cerebellum, bilateral fusiform gyrus, bilateral frontal lobes, bilateral precuneus, and midbrain showed significant negative correlations with BMI, after adjusting for age, lifetime alcohol intake, history of hypertension, and diabetes mellitus (Fig. 11) [5]. In addition, the regional gray matter volume of the bilateral inferior frontal gyri, bilateral posterior lobe of the cerebellum, bilateral frontal and temporal lobes, bilateral thalami, and bilateral caudate heads showed significant positive correlations with BMI, after adjusting for age, lifetime alcohol intake, history of hypertension, and diabetes mellitus in men. However, biological significance of these results remained unknown.

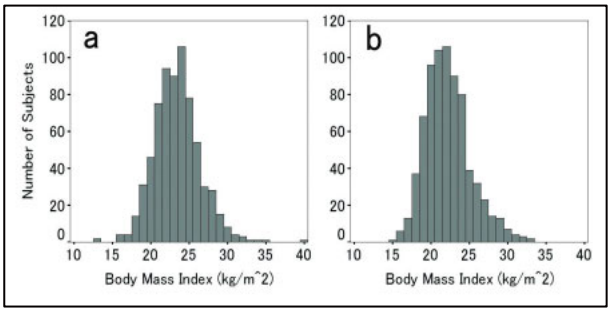

Fig. 10. Histograms of BMI in male and female

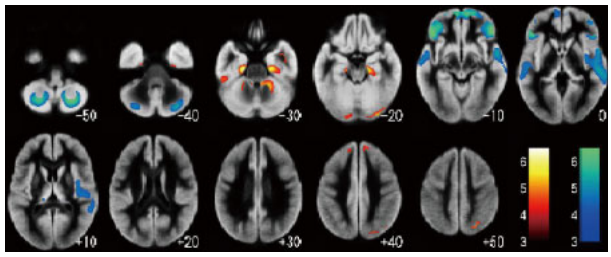

Fig. 11. Brain regions that showed correlations between gray matter volume and body mass index (BMI). Red and blue color indicated negative and positive correlations, respectively.

3.4.4. Mental status

Depression is one of the most common mental disorders in the elderly. There are subjects who have significant depressive symptoms but do not coincide with criteria for major depression. However, the symptoms of the subjects areassociated with deterioration of physical diseases and physical functions, higher risk of mortality, higher risk of suicide and thus, this state is considered as clinically important depression syndrome for the elderly and is defined as "sub threshold depression (sD)".

We defined sD as the subjects with Geriatric Depression Scale score of 15 and higher and Mini Mental State Examination of 22 or higher, and do not fulfill the criteria of major depressive disorder (MDD) in Diagnostic and Statistical Manual for Mental Disorders IV. We collected brain MRI of 34 elder subjects with sD (15 male, 9 female) and 109 age-matched normal subjects, and analyzed the difference in regional gray matter volume between these two groups.

Male subjects with sD had significantly smaller volumes of the medial part of bilateral frontal lobes and the right precentral gyrus than normal male subjects [6] (Fig. 12), while there are no significant volume differences between two female groups. These regions were known to be shown also in elderly MDD, although volume reduction in the hippocampus was additionally shown in MDD. Hippocampus may relate to the severity of the depression symptom. Our study reveals that even community-dwelling sD shows similar volume reduction to that in MDD, although they do not fully coincide with criteria for MDD.

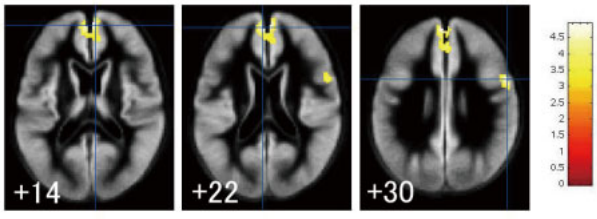

Fig. 12. The brain areas in which gray matter volume decreases in sD compared to age-matched control.

3.5. Automatic detection of ischemic lesion from 3-D MRI data

White matter hyperintensity areas（HIA）on T2 weighted MR images (T2WI) are known as ischemic changes and frequently observed in elderly people (Fig.13). The changes are related not only to age but also to cerebro-vascular risk factors, such as hypertension. However, the clinical significance of the changes in the healthy, neurologically non-diseased elderly people is not clarified yet. Moreover, the HIA on T2WI are usually rated using scoring system such as Fazekas by the radiologists. However, the rating with visual inspection is subjective and there are inconsistencies between radiologists. Furthermore, the scoring is time consuming and it is difficult to deal with a large number of samples. At present time, there is no sophisticated software for automatic detection system with high accuracy. In the present study, we aimed to develop an objective and reliable evaluation algorism by modification of the software (tissue classifier) which can classify the brain into different tissue compartments using artificial neural network (ANN) (Fig. 14).

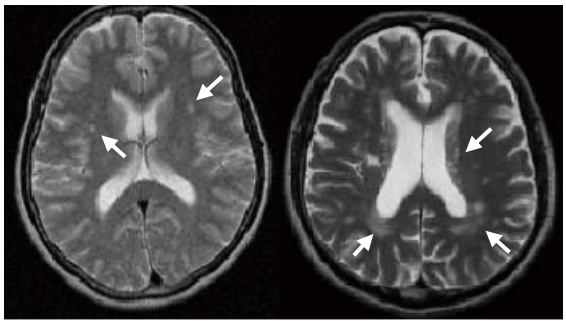

Fig. 13. Ischemic lesions in the white matter
Left: Slight spotty lesion (55-yr-old male) (arrows). Right: Moderate to severe lesions around the ventricle (arrows).

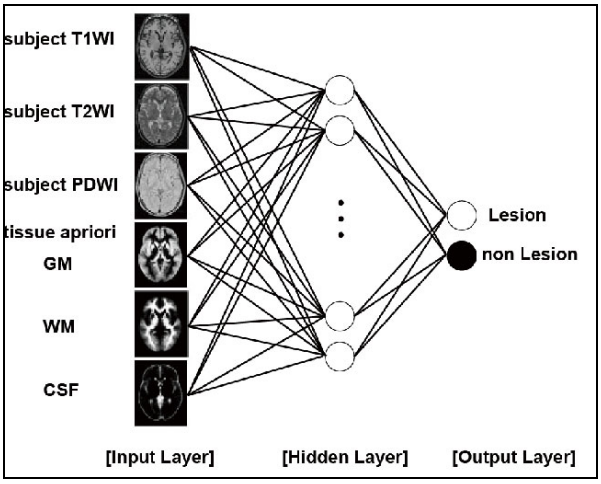

Fig. 14. Neural networks system for automatic detection of ischemic lesions.

Twelve elder subjects (male=6, female=6, Age=72.8+/-4.4 yrs.) with moderate HIA on T2WI were selected from Japanese Brain Database of AOBA. Subject who has history of neurological disorder, major infarction, brain tumor, traumatic lesion as well as lacunar infarction was excluded from the study. T1 weighted images and T2WI/Proton density weighted images were obtained from each subject.

All images were transferred to workstation and processed using general analysis software package developed at Montreal Neurological Institute. On the preprocessed images, T2WI HIA were (A) manually defined by well-trained neuroradiologist and (B) labeled automatically using multispectral classification technique based on artificial neural network (ANN) algorithm. We put 6 kind of information into input layer, that is, T1, T2, PD weighted MR images, and a priori anatomical information of gray matter, white matter and CSF. As output layer, we get binary image. Lesion voxel is marked 1 and non lesion voxel is marked zero.

We tuned ANN based classifier for the purpose of detection of subcortical ischemic lesions. An optimum parameter for training the classifier was searched. A parameter such that proportion of tag points=2% / # of tag points=5000 was thought to be tentative best one. Classification using above parameter, false positive rate and kappa statistics showed relatively good, however sensitivity showed relatively low [7].

Figure 15 shows the performance of this system. Well trained neuro-radiologists made gold standard data by manual tracing of the lesion and then accuracy of the automated system was evaluated using the standard data. Yellow indicates true positive, green false negative, blue false positive, respectively. Although false negative rate was relatively high, accuracy of this system was acceptable for the purpose of our study.

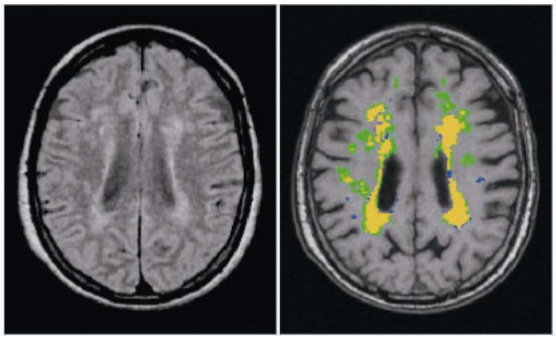

Fig. 15. A sample image of proton density weighted image (left) and white matter ischemic lesions superimposed on T1WI (right). Yellow: true positive, green: false negative, blue: false positive.

Figure 16 shows number of subjects for each decade that had white matter ischemic lesions and analyzed in this study. Each age and sex group had more than 80 subjects. We made a ischemic lesion map using the algorism for each subject and lesion probabilistic map.

Figure 17 shows probabilistic lesion map for each decade, which was made by summing up lesion map of a subject belonging to the same age group. The lesion distributed along the ventricle and total lesion volume increased with increasing of age.

Then, we made voxel-based correlation analysis between lesion probability and risk factors. Among the risk factors, only hypertension correlated with the ischemic lesion in the frontal part of the brain (data not shown).

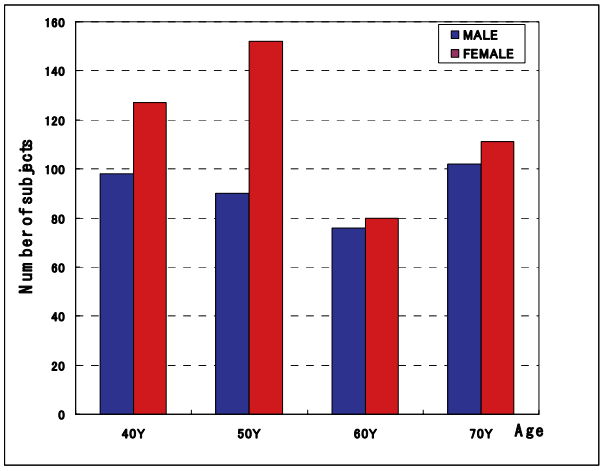

Fig. 16. Number of subjects in each decade and sex

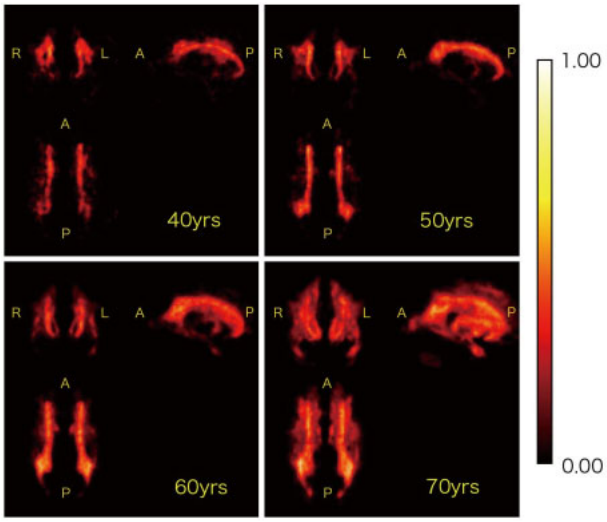

Fig. 17. The probabilistic lesion map for each decade (40th, 50th, 60th and 70th year-old)
Upper left: coronal view, upper right: sagital view, lower left: axial view.

4. Summary and Further Research Plan

We made the following research plans at the start of this global COE (Center of Excellence) project.

Research plan (1): Correlation between gray matter volume and cerebrovascular risk factors. Results and progress

As described in the result section (**3.4.1-3.4.4**), we found negative correlations between gray matter volume and hypertension, lifetime alcohol intake, obesity (BMI), mental status (subthreshold depression). However, we could not find any correlation with diabetes mellitus, hypercholesterolemia and smoking habit. We will continue the correlation analysis with other factors.

Research plan (2): Quantitative measurement of ischemic lesions in the white matter and correlation analysis between the lesion and global and regional gray matter volume.

Results and progress

We developed an algorism for fully automated detection of ischemic white matter lesion with high accuracy. Using this system, we made lesion probabilistic map for each decade and clarified that the lesion localize around the ventricles and the lesion volume increased with age.

Next step is to analyze correlation between total lesion volume and global or regional gray matter volume and to analyze correlation between the regional lesion probability and regional gray matter volume.

Research plan (3): Longitudinal analysis of brain volume change during aging

Results and progress

We analyzed brain volume change during aging. However, our data were obtained from the cross sectional study and therefore, these data do not represent age-related changes in strict meaning. To overcome this problem, we made longitudinal study design. We collected brain MRI 6-8 years ago and constructed database (Aoba-1). From the database, we selected 600 subjects and asked them to take brain MRI again and co-operate our research project. Among them, 380 subjects accepted our proposal. Then, we took their MRI from April to August, 2007. Preliminary analysis for 200 subjects revealed that there are sex differences in gray matter loss with age as shown in results section **3.3**. We also found that white matter volume increased at earlier age and then went down thereafter. The result from cross sectional study (Fig. 4) suggested the same tendency, but it was not clear. We are continuing the analysis using with full data set (Aoba-2). The concern is whether the rate of gray matter loss per year is the same or different

between cross sectional design and longitudinal design, and to find regional differences of brain volume change in longitudinal study.

Research plan (4): New research plan using a 3.0 T MRI system

MRIs we collected were taken using 0.5 Tesla MRI system, and therefore image quality is not quite good, although it was acceptable for the analysis we did. We are going to buy a 3.0 Tesla MRI system and install it by the end of March, 2008. Using this new system, we can get images with high signal to noise ratio and high tissue density resolution. The next plan is to clarify development process of the brain in younger subjects. For that purposes, we are planning to collect and analyze brains in children with primary school age.

5. Prospect of This Imaging Project

Aging and senescence of the society is rapidly progressing in Japan. The aging medicine is becoming more and more important to overcome age-related diseases such as Alzheimer's Disease. In particular, neuroimaging for human brain is becoming important, because it will be beneficial for the early diagnosis and management of age-related brain diseases. In this project, we aimed to understand normal (healthy) brain aging through the analysis of human brain MRI and to clarify the risk factors which accelerate brain aging. Finally, we intended to give the results back to clinical medicine and apply it to the prevention of brain aging.

References

[1] Sato K, Taki Y, Fukuda H, Kawashima R. Neuroanatomical database of normal Japanese brains. *Neural Networks* **16**, 1301-1310, 2003.

[2] Taki Y, Goto R, Evans A, et al. Voxel-based morphometry of human brain with age and cerebrovascular risk factors. *Neurobiol Aging* **25**, 455-63, 2004.

[3] Good CD, Johnsrude I, Ashburner J, et al. Cerebral asymmetry and the effects of sex and handedness on brain structure: a voxel-based morphometric analysis of 465 normal adult human brains. *Neuroimage* **14**, 685-700, 2001.

[4] Taki Y, Kinomura S, Sato K, et al. Both global gray matter volume and regional gray matter volume negatively correlate with lifetime alcohol intake in non-alcohol-dependent Japanese men: A volumetric analysis and a voxel-based morphometry. *Alcohol Clin Exp Res* **30**, 1045-1050, 2006.

[5] Taki Y, Kinomura S, Sato K, et al. Relationship between body mass index and gray matter volumes in 1428 healthy individuals. *Obesity*, 2008 (in press).

[6] Taki Y, Taki Y, Kinomura S, Awata S, et al. Male elderly subthreshold depression patients have smaller volume of medial part of prefrontal cortex and precentral gyrus compared with age-matched normal subjects: A voxel-based morphometry. *J Affect Disorders* **88**, 313-320, 2005.

[7] Kinomura S, Lerch L, Zijdenbos AP, et al. Automatic detection of subcortical ischemic lesion from 3-D MRI data in the aged brain. In *10th Annual Meeting of the Organization for Human Brain Mapping*, Budapest, Hungary, 2004.