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Original Article

Brain Development Measured With MRI in Children With Down Syndrome Correlates With Blood Biochemical Biomarkers

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ABSTRACT

Background: Down syndrome (DS) is a neurodegenerative disease with unknown mechanisms. β-Amyloid peptide (Aβ) and tau protein (Tau) are known to play a role, while vitamin A (VA) has an effect on normal neurological function. In a case-control study, we quantitatively evaluated whole brain and hippocampal volumes of DS children and analyzed the correlation of hippocampal volumes with blood levels of Aβ, Tau and VA.

Methods: All subjects underwent magnetic resonance imaging (MRI) of the brain. The whole brain and hippocampal volumes were quantitatively analyzed using voxel-based morphometry (VBM) and stereology respectively. The blood levels of Aβ, Tau, and VA were detected by enzyme-linked immunosorbent assay and high-performance liquid chromatography, respectively.

Results: Thirty DS children and twenty healthy controls were recruited. Whole brain and hippocampal volumes were significantly smaller in individuals with DS than in healthy controls. In both groups, whole brain and hippocampal volumes increased in accordance with age. The results of correlation analysis suggested that Aβ42/Aβ40 and VA are associated with hippocampal volume in DS patients.

Conclusion: DS children exhibited neurodevelopmental defects, even at an early age. Aβ42/Aβ40 and VA may affect hippocampal volume in DS patients.

Introduction

Down syndrome (DS) is a common genetic disorder that has cognitive consequences. People with DS exhibit age-related neurodegeneration and tend to develop Alzheimer disease (AD).[1] Senile plaques and neurofibrillary tangles (NFTs), common pathologic changes in AD, have been detected in the brain of DS adults older than 30 to 40 years.[2] β-Amyloid peptide (Aβ), a neurotoxic hydrolyzed product of amyloid precursor protein, plays a role in senile plaque formation and relates to cognitive and mental behavior disorders.[3] Excessive phosphorylation of tau protein (Tau) is involved in the formation of NFTs. *In vivo*, Aβ is composed of two main forms: Aβ40 and Aβ42, with Aβ42 forming aggregates and having a stronger neurotoxic effect than Aβ40. Aβ oligomers can induce tau aggregation.[4]

Vitamin A (VA) is a necessary micronutrient and is essential for maintaining normal functioning of the nervous system.[5] VA plays a role in the deposition of Aβ through its nuclear receptor.[6] There have been no studies of trends in the neurodevelopment with age in DS children, nor studies of the correlation of Aβ, Tau and VA levels with hippocampal volumes in DS children.

The purpose of this study was to quantitatively analyze brain and hippocampal volumes in DS children as compared with those in healthy controls (HC), to determine age-dependent changes in hippocampal volumes, and to analyze the correlation of hippocampal volumes with levels of Aβ42/Aβ40, Tau, and VA in DS children.

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Material and Methods

Participants

This was a case-control study. The DS children were admitted to the Children's Hospital of Chongqing Medical University (CHCMU) from August 2014 to March 2015. The inclusion criteria were as follows: Chinese Han children, a clinical diagnosis of standard karyotype DS by karyotype analysis and no systemic disease manifestations, including but not limited to serious heart defects and gastrointestinal malformations. The exclusion criteria were: complications including serious congenital heart disease, epilepsy, alimentary tract malformation, malnutrition, infectious diseases, and hematological diseases with abnormal bleeding or coagulation function; perinatal abnormality including prematurity, anoxia asphyxia, pathologic jaundice; inability to complete magnetic resonance imaging (MRI) due to metal objects in the body; medication or dietary supplements containing VA within six months of the study. The HC were randomly recruited from kindergartens and schools cooperating with CHCMU. Inclusion criteria for HC were: Chinese Han children; no developmental malformations, no special facial features, no special skin texture, no intellectual disability, no physical or intellectual developmental delay, no family history of genetic or chromosomal disorders; willingness to participate in the study with a signed informed consent form by the guardian; willingness to provide blood samples by venipuncture and to take head MRI examinations. Exclusion criteria for HC were: congenital heart disease, hematological diseases, or other diseases with bleeding tendency or coagulopathy, infectious diseases, epilepsy, gastrointestinal malformation, or malnutrition; inability to complete the MRI due to metals in the body (for example, internal fixation of fracture). This study conformed to the tenets of the Declaration of Helsinki and was approved by the ethics committee of CHCMU. Signed informed consent forms were obtained from all the participants' parents or guardians. The study protocol and sample size recruited in each step was described in Fig 1.

Cranial imaging examination

MRI acquisition

Cranial images were obtained using a Signa Propeller HD 1.5 T Echospeed Superconducting magnetic resonance apparatus (General Electric, Fairfield, CT), equipped with an eight-channel phased-array head coil. 3DT1W1 thin layer scanning was applied. After three plane positioning, oblique coronal scan was achieved using fast interference phase gradient echo, pulse sequence (T1WI). The parameters were as follows: repetition time = 11.1 ms, echo time = 5 ms, flip angle = 20° and layer thickness = 1.0 mm. Continuous scanning was performed (FOV = 240 mm × 240 mm, matrix = 256 × 256, Nex = 1). In all cases, trained imaging specialists performed the scanning, using uniform scanning parameters.

Calculation of hippocampal volume

The hippocampal volume was quantitatively calculated using the unbiased stereology based on Cavalieri theory.[7] The subjects were scanned using the above parameters to obtain a clear-edged hippocampal image, with a thickness of 1.0 mm. A transparent measuring point box was randomly stacked on the hippocampal image, and the number of points $\Sigma P(HF)$ in the region of interest was then counted. The total volume of the

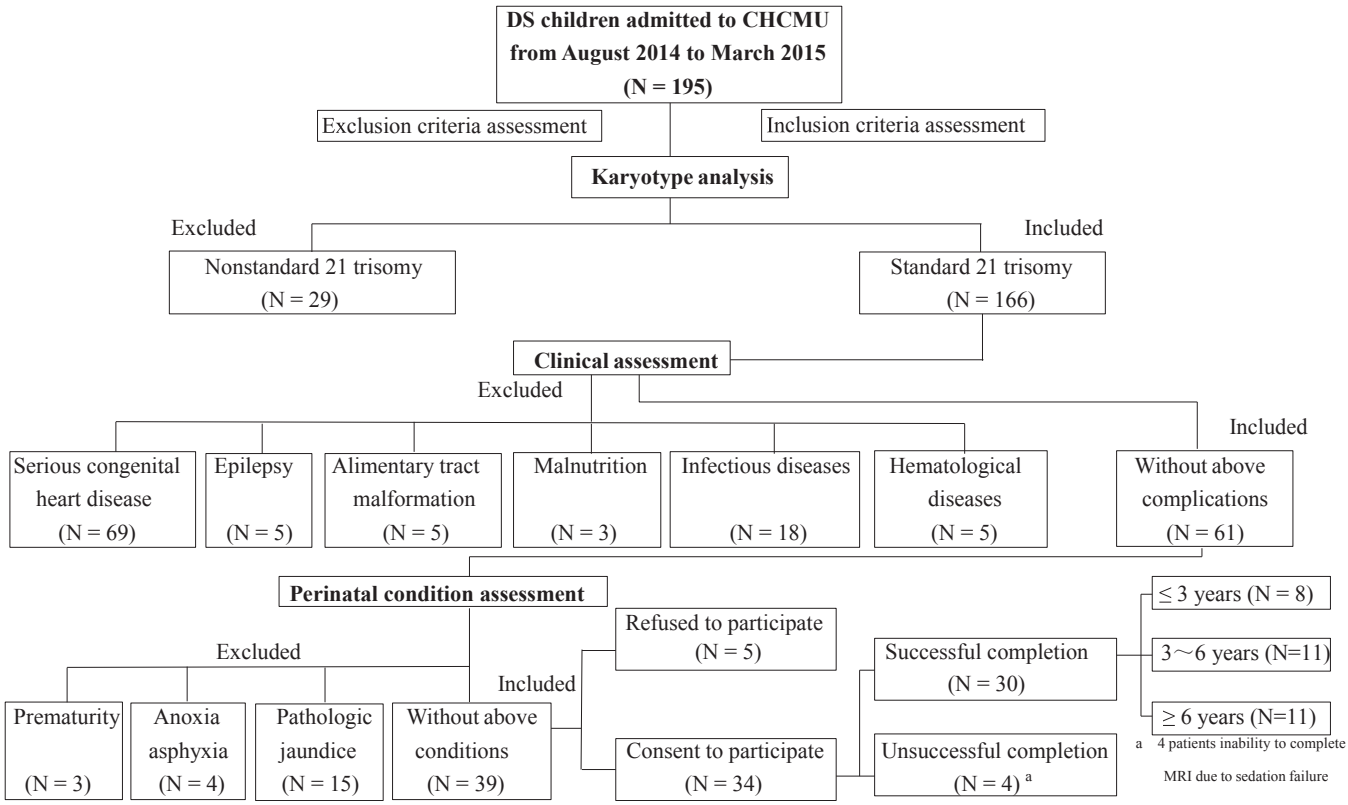

FIGURE 1. Study protocol and sample size recruited in each step.

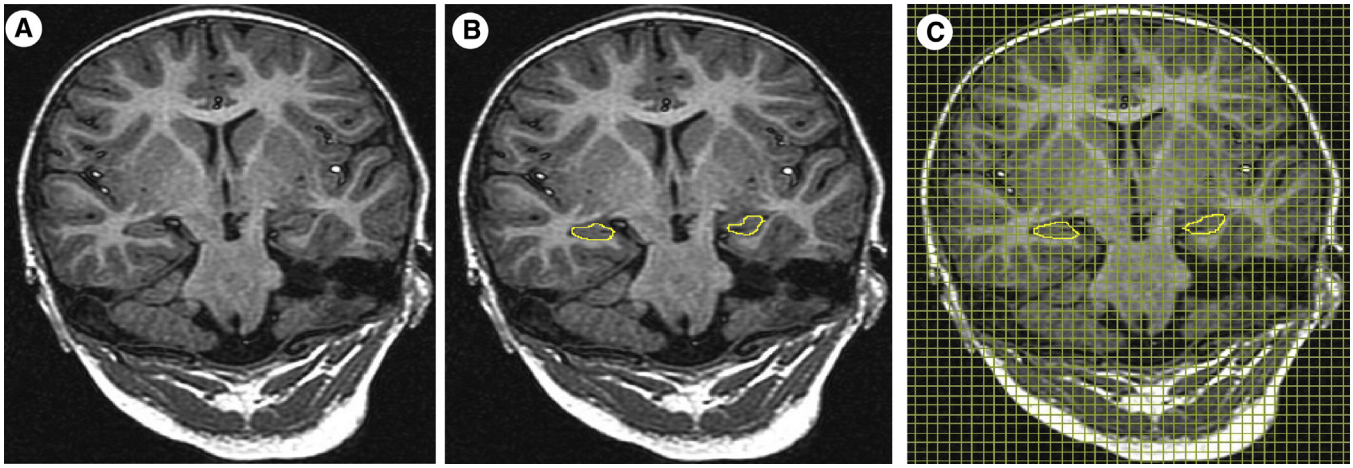

FIGURE 2. Quantitative measurement of hippocampal volume by stereology.
(A) Read the MRI images containing hippocampal.
(B) Outline the boundary of the hippocampal.
(C) Stack unbiased body frame on the boundary of the hippocampal randomly.

hippocampal was calculated according to Cavalieri theory, as follows:

$$V1(HF) = t \times a(p) \times \sum P(HF)$$

V1 (HF) is the first layer of hippocampal volume, t is the slice thickness (1 mm), *a (P)* is the area represented by each measuring point (1 mm^2), and $\sum$ *P (HF)* is the measuring points in the hippocampal section. The whole volume was calculated by superposition of *V*1, *V*2, and *V*3 (Fig 2).

Calculation of the whole brain volume

The captured images were processed with voxel-based morphometry (VBM) software in Statistical Parametric Mapping 8 (SPM8) software toolbox under the running environment of MatLab. We performed the preprocessing steps using the Computational Anatomy Toolbox (CAT12, http://dbm.neuro.uni-jena.de/cat/) of Statistical Parametric Mapping 8 (http://www.fil.ion.ucl.ac.uk/spm) with the default setting. The analysis incorporates the following preprocessing steps: (1) tissue segmentation, (2) spatial normalization, (3) modulating, and (4) smoothing with a Gaussian kernel size of 8 mm.[8]

Blood collection and laboratory measurements

Blood samples were collected in K2-EDTA tubes and dry tubes for plasma and serum respectively. The samples were stored in the dark at −80°C and thawed at room temperature before determining the level of Aβ42, Aβ40, Tau, and VA.

Plasma Aβ42, Aβ40, and Tau were measured by enzyme-linked immunosorbent assay using a commercial kit (Invitrogen, Carlsbad, CA). The final absorbance was determined at 450 nm using a microvolume spectrophotometer system (BioTek, Winooski, VT). Serum VA concentration was measured by high-performance liquid chromatography based on previously described methods.[9]

Statistical analysis

Values having normal distributions were expressed as the mean ± standard deviation (SD). Categorical variables were expressed as percentages. Multivariate regression analysis was used to adjust the effect of age and gender on whole brain volume and the effect of age, gender and whole brain volume on hippocampal volume. In the multivariate analysis, the brain volume was standardized by the mean value of all subjects' brain volume. Between-group comparisons of age were performed using an independent t test. The Chi-square test was conducted for comparisons of categorical variables. Spearman's correlation analysis was conducted to discern correlations between quantitative variables. A statistically significant difference was denoted by $P < 0.05$. All statistical analyses were performed using SPSS 22 software (IBM, NY).

Results

In total, 30 children with DS and 20 HC were studied. The youngest subjects in both groups were aged 0.33 years while the oldest individuals were 15.67 years and 14.10 years in the DS and HC groups, respectively. There was no statistical difference in the mean age and gender comparison of the subjects in DS and HC ($t = -0.39$, $P = 0.70$; $\chi^2 = 0.14$, $P = 0.71$) (Table 1).

Whole brain volumes were significantly smaller in DS than HC ($\beta = -0.57$, $P = 0.0099$). The right, left and whole hippocampal volumes were remarkably smaller in DS than HC as well ($\beta = -0.49$, $P = 0.0016$, $\beta = -0.48$, $P = 0.0016$, $\beta = -0.49$, $P = 0.0015$) (Table 2).

In both DS and HC, whole brain volumes, right, left and whole hippocampal volumes increased with age remarkably ($\beta = 0.15$, $P < 0.0001$; $\beta = 0.14$, $P < 0.0001$; $\beta = 0.15$, $P < 0.0001$; $\beta = 0.15$, $P < 0.0001$) (Table 3).

The results of the correlation analysis showed that right, left and whole hippocampal volumes in DS were negatively correlated with Aβ42/Aβ40 ($r = -0.82$, $P = 0.00$; $r = -0.83$, $P = 0.00$, $r = -0.83$, $P = 0.00$) and positively correlated with VA level ($r = 0.40$, $P = 0.03$; $r = 0.40$, $P = 0.03$; $r = 0.41$, $P = 0.03$) (Table 4).

TABLE 1.
Comparison of Age and Gender Composition Between DS and HC Groups

Groups\Indexes	DS (n = 30)	HC (n = 20)	t/χ^2	P
Age*	5.31 ± 4.01	5.76 ± 3.91	−0.39	0.70
Gender (M/F)†	9/21	7/13	0.14	0.71

DS, Down syndrome; HC, healthy controls.
* Continuous data are shown as mean ± standard deviation (SD). Independent t test was used to compare the variables.
† Data are shown as frequency. Chi-square test was used to compare the variables.

TABLE 2.
Comparison of the Whole Brain Volume and Hippocampal Volume Between DS and HC Group After Data Standardization

Volume\Variable	β	95% CI		t	P
Whole brain volume (HC ref.)*	−0.57	−0.99	−0.14	−2.69	0.0099
Right hippocampal volume (HC ref.)†	−0.49	−0.78	−0.20	3.36	0.0016
Left hippocampal volume (HC ref.)†	−0.48	−0.77	−0.19	3.36	0.0016
Whole hippocampal volume (HC ref.)†	−0.49	−0.78	−0.20	3.37	0.0015

HC, healthy controls.
* Comparison of the whole brain volume between DS and HC adjusted for age and gender.
† Comparison of the right, left and whole hippocampal volume between DS and HC adjusted for age, gender and whole brain volume.

Discussion

DS is a common chromosomal disorder that is associated with cognitive impairment. Its approximate incidence is 1/600 to 1/1000.[10] DS patients show characteristic neuropsychologic features, including but not limited to language delay and memory loss.[11] Moreover, DS patients show a tendency to develop AD after middle age.[2,12] Therefore, studies of morphologic and pathologic changes linked to neurodegeneration in DS have focused mainly on adults. Related studies showed that both whole brain and hippocampal volumes of DS adults were significantly smaller than those of HC.[13–15] As these studies did not include DS children, they could not clarify whether the smaller hippocampal volume in DS was caused by the disorder itself, age-related neurodegeneration, or the superimposed effect of both factors.

A previous study showed the hippocampal volume was smaller in DS children than in normal individuals.[16] However, this study was limited by a small sample size (n = 6) and the use of a low-resolution MRI. Another study of 16 DS children and 15 HC showed that whole brain, right and left hippocampal volumes of DS were significantly smaller than those of HC.[17] The results of this study benefited from a slightly larger sample size than the former one, as well as the use of accurate quantitative neuroimaging methods. However, all the subjects included were older than five years, while the first five years of life are the most important stage as regards to brain development. Our study recruited 30 DS children, including five children younger than three years. The youngest subject was just 0.33 years old. Although the resolution of the 1.5 T scanner we used was less than 3.0 T, the DS and HC were examined with the same scanning method; VBM and unbiased stereology were used to quantitatively analyze the brain and hippocampal volumes. Our data confirmed that whole brain and hippocampal volumes of DS children were remarkably smaller than those of HC, even in the case of including very young patients.

Previous studies reported that brain and hippocampal volumes declined with increasing age in adults with DS. However, a controversy surrounded whether the volume reduction preceded clinical manifestations of neurodegeneration.[13,14,18] The nervous system continues to develop throughout childhood, during which both brain volume and weight gradually increase and brain function improves. However, there are few studies on the changes of the brain volume with aging of DS children. In this study, we recruited both of DS and HC children of different ages; our results indicate that whole brain volumes and hippocampal volumes increase with age in both DS and HC. Combined with the findings from previous studies, we conclude that the trend of brain and hippocampal volume changes with age varies in different stages. The volume mainly increases in childhood and decreases in adulthood. Our study will improve the understanding of the development trend of the brain and hippocampal volume in DS children.

TABLE 3.
The Trend of Brain and Hippocampal Volume Growth With Age

Volume\Variable	β	95% CI		t	P
Whole brain volume (HC ref.)*	0.15	0.09	0.20	5.44	<0.0001
Right hippocampal volume (HC ref.)†	0.14	0.10	0.19	6.57	<0.0001
Left hippocampal volume (HC ref.)†	0.15	0.10	0.19	6.73	<0.0001
Whole hippocampal volume (HC ref.)†	0.15	0.10	0.19	6.68	<0.0001

HC, healthy controls.
* The trend of whole brain volume with age adjusted for gender.
† The trend of right, left and whole hippocampal volume with age adjusted for gender and whole brain volume.

TABLE 4.
Correlation Analysis of Hippocampal Volume and Blood Biochemical Parameters in DS Children

Variable		Aβ42 (pg/mL)	Aβ42/ Aβ40	Total Tau (pg/mL)	VA (μmol/L)
The right hippocampal volume (mm^3)	***n***	30	30	30	30
	r	−0.09	−0.82	0.17	0.40
	P	0.62	**0.00**	0.37	**0.03**
The left hippocampal volume (mm^3)	***n***	30	30	30	30
	r	−0.09	−0.83	0.15	0.40
	P	0.62	**0.00**	0.42	**0.03**
The whole hippocampal volume (mm^3)	***n***	30	30	30	30
	r	−0.10	−0.83	0.14	0.41
	P	0.61	**0.00**	0.45	**0.03**

Aβ42, β amyloid peptide 42; Aβ40, β amyloid peptide 40; Tau, tau protein; VA, vitamin A.

Neurotoxic senile plaques are composed mainly of Aβ.[19,20] Senile plaques in individuals with DS show a greater density and distribution in the brain than occurs in AD patients.[21,22] Compared with Aβ40, Aβ42 is more hydrophobic and neurotoxic and more likely to form oligomers;[23] therefore Aβ42 appeared to play a more important role than Aβ40 in neurodegeneration.[24] Given differences between individuals in total protein load, measurements of Aβ42/Aβ40 are more valuable than using an absolute value of Aβ42. Therefore, in this study, we focused on the level of Aβ 42 and Aβ42/Aβ40. The results revealed no statistically significant correlation between hippocampal volumes and Aβ42 in DS. However, hippocampal volumes showed a significant negative correlation with Aβ42/Aβ40. We speculate that a high level of Aβ42/Aβ40 may be more sensitive than Aβ42 to reflect Aβ's negative effect on the nervous system of DS.

In addition to senile plaques, NFTs are a characteristic pathologic change in the DS brain which are formed by highly phosphorylated Tau. Our study showed that hippocampal volumes did not correlate with the plasma total Tau in DS. Previous research demonstrated that calcipressin and DYRK1A were phosphorylation regulators of Tau which were located on chromosome 21.[25,26] Murphy et al. reported that NFTs found in the brain of DS patients formed after Aβ deposition.[27] We attribute the absence of a correlation between hippocampal volumes and plasma total Tau in the present study to two factors: First, the DS included in the study consisted of children, ranging in age from 0.3 to 15.7 years, and Aβ deposition and subsequent NFTs formation is a gradual process that occurs over time. Therefore, the total Tau level may not have accumulated enough to show correlation with hippocampal volumes. Second, according to the gene dosage effect, the level of phosphorylated Tau may be increased in DS patients, but we measured only the level of total Tau. Thus the level of phosphorylated Tau may be more important than that of total Tau with regards to DS.

Previous research confirmed that VA played a major role in the hippocampal by maintaining its structure and function.[28,29]

Although the relationship between VA and the hippocampal has been extensively reviewed, no studies have examined the correlation between VA level and hippocampal volumes, particularly in a DS population. The present study showed that hippocampal volumes in DS children were positively correlated with VA levels. We suggest that VA may promote hippocampal development of DS children, although the exact mechanism remains unknown.

Limitations

Small sample size was one limitation of this study. A replication study with a larger sample size should be meaningful to reflect the correlation with variables based on more comprehensive statistical methods. Using a 1.5 T scanner was another limitation that should be replaced by a 3.0 T scanner in the further study to gain higher resolution images. Single total Tau measurement should be replaced by phosphorylated Tau detection in subsequent studies.

Conclusions

Individuals with DS exhibit neurodevelopmental defects from childhood. Aβ42/Aβ40 and VA have negative and positive correlations to hippocampal volumes in DS respectively.

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