Brain metabolic profile obtained by proton magnetic resonance spectroscopy HMRS in children with Down syndrome

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Abstract

Purpose: Down syndrome (DS), or trisomia 21, is one of the most common autosomal mutations, with mental impairment as the constant symptom. Proton magnetic resonance spectroscopy (^{1}HMRS) allows evaluation of this metabolism in DS children. The study objective was the morphological evaluation of the brain in magnetic resonance imaging (MRI) and assessment of the metabolic profile obtained by HMRS in children with DS.

Material and methods: The study involved 34 children, including 14 with DS, aged 7-17 years. All of them were patients of the Department of Pediatric Neurology and Rehabilitation, Medical University of Białystok, and of its Outpatient Clinic. Age-matched healthy children (n=20) served as control. MRI scans of the head were performed in DS children using a 1.5T MR scanner in standard conditions, in three planes (saggital, axial and coronal), in T1, T2, PD and FLAIR series. HMRS investigations were also conducted to assess metabolic changes in the frontal lobes. Such metabolites as Glx, NAA, Cho, mI and GABA were determined in both temporal lobes with reference to the internal marker Cr. Results were compared to the control group.

Results: The MRI revealed no structural changes in children with DS. We found a decrease in Glx/Cr, NAA/Cr, Cho/Cr and mI/Cr ratios in our DS patients as compared to the control group. The differences for the first two markers were statistically significant. However, no differences were found between GABA/Cr ratio in the two frontal lobes in patients with DS as compared to the control group.

Conclusions: Our findings seem to confirm the abnormal metabolism of stimulatory amino acids with developmental disorders and "precocious brain aging" in children with DS.

Key words: Down syndrome, magnetic resonance spectroscopy, frontal lobes.

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Introduction

Down syndrome (DS) belongs to the most common autosomal genome mutations [1,2]. Disorders observed in DS are due to the presence of an additional chromosome 21 or its segment containing the so-called "critical region" [3,4]. Mental impairment is a constant symptom [5,6]. Further impairment of the cognitive functions that begins at the age of 35-40 is ascribed to dementia. There is evidence for a common genetic and pathophysiological background of dementia in DS and Alzheimer disease (AD). One of the early-onset AD genes is found in chromosome 21. Single base mutation in the amyloid precursor protein (APP) gene in chromosome 21 leads to accumulation of amyloid protein in senile cells. A similar mechanism of "precocious aging" of the brain has been observed in DS. Magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (^{1}HMRS) provide new possibilities for the evaluation of brain "precocious aging" in DS. ^{1}HMRS allows non-invasive *in vivo* determination of brain metabolite content, or rather of the proportions of the metabolites in the respective structures of the brain. Spectroscopy facilitates assessment of such metabolites as N-acetylaspartate (NAA), choline (Cho), creatinine (Cr), myoinositol (mI) and gamma-amino butyric acid (GABA), which are known to play a key role in the function of the nervous system, e.g. in the processes of memory and learning. ^{1}HMRS is used in the diagnosis of metabolic disorders of the central nervous system, ischemic-hypoxemic conditions, brain tumors and Alzheimer disease [7-10]. Spectroscopy is also applied for DS diagnostics in association with brain "pre-

Figure. 1. **[1]HMRS spectrum in a 12-year-old boy with Down syndrome (voxel vol. 2x2x2 cm3) in the left and right frontal lobe**

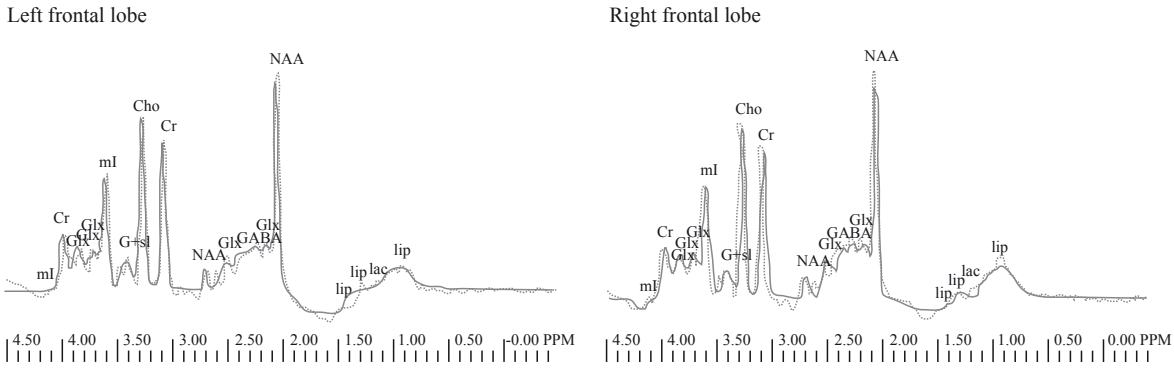
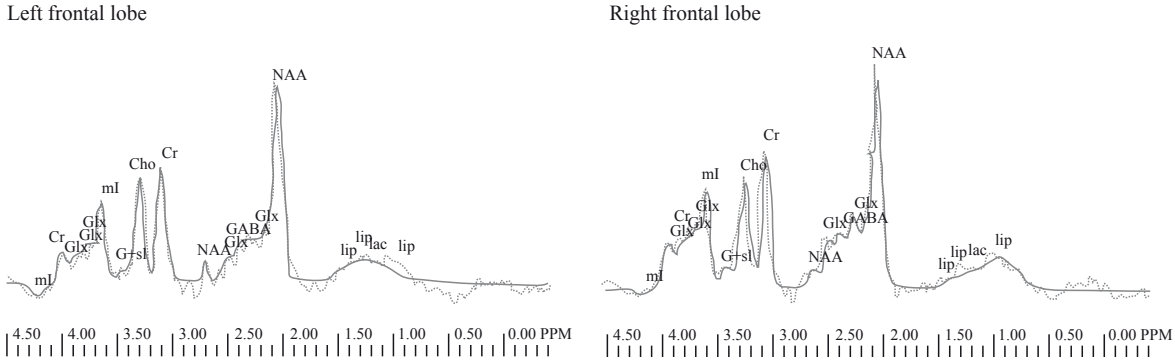


Figure 2. **[1]HMRS spectrum in a 13-year-old healthy boy (voxel vol. 2x2x2 cm3) in the left and right frontal lobe**



[1] HMRS spectra did not differ between the right and left lobe

cocious aging" processes and symptoms of dementia of the Alzheimer type [11]. The available literature is not abundant and refers to adults with Down syndrome. Most authors have conducted measurements of the respective metabolites in the hippocampal region, in the basal ganglia, and in the parietal and occipital lobes [10-12]. Berry et al. [13] showed a significant increase in the mI level in the basal ganglia (striatum) in DS children as compared to the control group.

In the present study, we decided to carry out similar measurements in the frontal lobes, i.e. in the structures never before assessed with respect to brain metabolite content.

Thus, the study objective was the morphological evaluation of the brain in magnetic resonance imaging (MRI) and assessment of the metabolic profile in the two frontal lobes in HMRS in children with Down syndrome.

Material and methods

Thirty-four children were recruited for the study, including 14 with DS (7 girls and 7 boys aged 6-17, mean 10.92±3.49) and 20 healthy children (11 girls and 9 boys aged 7-17, mean 11.78±3.92). All of them were patients of the Department of Pediatric Neurology and Rehabilitation, Medical University of Białystok, and of its Outpatient Clinic. MRI scans of the head were performed in DS children using 1.5T MR scanner in standard conditions, in three planes (saggital, axial, coronal), in T1, T2, PD and FLAIR series. In all the cases, [1]HMRS investigations were also conducted to assess the metabolic profile in the frontal lobes. NAA, Cho, Glx, mI and GABA markers were determined with reference to the internal marker Cr. The voxel volume was 2x2x2 cm^3. The results were compared to the control [1]HMRS findings.

Results

The imaging of the brain revealed no structural changes in children with DS as compared to the control group.

Fig. 1 and *Fig. 2* present [1]HMRS spectrum in the two frontal lobes in a patient with Down syndrome and in a healthy control subject.

Table 1. presents the proportions of metabolites in the two frontal lobes in children with Down syndrome and in the control group.

The frontal lobes of DS children showed reduced NAA/Cr, Glx/Cr, Cho/Cr and mI/Cr ratios. The differences between the ratios of the first two markers to creatine were statistically sig-

Table 1. **Metabolite proportions in the two frontal lobes in children with Down syndrome (n=14) and in the control group (n=20)**

Index	Down syndrome	Control	p value
	(n=14)	(n=20)	
Glx/Cr	2.17 ± 0.97*	3.38 ± 1.41*	p= 0.011
NAA/Cr	1.66 ± 0.56	1.95 ± 0.5	p= 0.0354
Cho/Cr	1.08 ± 0.23	1.15 ± 0.23	NS
mI/Cr	1.52 ± 0.36	1.75 ± 0.47	NS
GABA/Cr	1.92 ± 0.19	1.99 ± 0.33	(-)

NAA – N-acetylaspartate; Cho – choline; Cr – creatinine; mI – myoinositol; Glx – glutamate-glutamine complex; GABA – gamma-amino butyric acid; NS – lack of statistical significance; Wilcoxon test; * The presented values are means of 28 and 40 estimations

nificant (*Tab. 1*). However, no differences were found between GABA/Cr ratios in the two frontal lobes in patients with DS as compared to the control group.

Discussion

We found a reduction in the NAA/Cr, Glx/Cr, Cho/Cr and mI/Cr ratios in both frontal lobes of DS children as compared to healthy controls. The differences for the first two markers were statistically significant. NAA, Glx and Cho belong to the stimulatory amino acids of the central nervous system. They play a significant role in stabilization and maintenance of the so called long-term potentiation (LTP), which is a learning and memory exponent observed in neurons *in vitro* [14]. It is believed that approximately 70% of neurotransmissions in the brain occur via stimulatory amino acids. During acute hypoxia of the whole brain or its respective structures, neurons release increased amounts of stimulatory amino acids, showing a strong destructive cytotoxic action. Stimulatory amino acids play a key role in the pathogenesis of psychotic disorders (anxiety, depression) and neurodegenerative diseases (epilepsy, stroke, post-traumatic brain damage, Alzheimer disease, Parkinson disease, Huntington chorea) [15,16]. We revealed a significant reduction in the Glx/Cr ratio in both frontal lobes in DS children. Glx is a neurotransmitter of the glutaminergic system, which is the major stimulatory system in the CNS [16,17]. It plays a key role in neuron maturation as it regulates the processes of proliferation and migration of neural precursors and immature neurons during brain development. It has an important function in learning and memory processes, and regulates pain signal transduction in the spinal cord and brain. According to Castillo et al. [18], Glx is also involved in mitochondrial metabolism, in detoxification processes and in the regulation of the activity of other neurotransmitters. The significant reduction in the Glx/Cr ratio observed in the current study in frontal lobes in DS children as compared to healthy controls indicates disorders in the system of stimulatory neurotransmitters and may suggest impaired neuronal maturation during brain development, and in consequence disorders in learning and memory processes.

We also found a significant drop in the NAA/Cr ratio in DS children. NAA is another stimulatory amino acid. It is found in neurons, neuroglial precursor cells and immature oligodendrocytes, and being an intracellular amino acid, it is considered to be a neuronal density marker. NAA is involved in many biochemical processes, e.g. in aspartate metabolism, lipid synthesis and osmotic cell regulation. Considering the correlation of NAA concentration with the number of cells and their metabolic efficiency, NAA has been regarded as a marker of metabolic fitness of neurons. According to Hsu et al. [19], there is some evidence of NAA involvement in the myelinization processes. Disturbances in the level of NAA have been observed in epilepsy, multiple sclerosis, amyotrophic lateral sclerosis, neurodegenerative diseases and brain ischemia [20,21]. ^{1}HMRS has been applied many times in studies concerning dementia of the Alzheimer-type. Hsu et al. in 2001 [19] described in dementic patients a reduction of NAA level and NAA/Cr ratio in the frontal, parietal and occipital lobes, as well as in the centrum semiovale and hippocampus. Valenzuela et al. [22] found an approximately 15% drop in NAA level, which is an early phenomenon and is not always associated with the structural changes visualized by MRI. Jessen et al. [23] based on the analysis of the NAA/Cr and Cho/Cr ratios in the brain regions undergoing degeneration in subsequent phases of dementia, revealed differences between these markers which confirmed the order of development of neurodegenerative changes (the median temporal lobe, the primary motor and sensory cortex). Correlations have also been found between changes in the level of NAA in spectroscopy and enhancement of Alzheimer-type pathology (number of amyloid plaques and neurofibril degeneration) [24].

In most patients with DS, symptoms of Alzheimer disease appear earlier, i.e. already around 40 years of age [25]. It has been also found that activity of the gene responsible for the production of amyloid is increased in DS patients. Dementia symptoms may be observed in 0-4% of DS patients under 30 and in 29-75% of cases between 60-65 years of age [26]. Patel et al. [27] showed more frequent occurrence of Alzheimer disease in patients with mental impairment, and especially with trisomia of chromosome 21. The neuropathological Alzheimer-type changes in DS patients suggest that the genetic defect in familial Alzheimer disease is also associated with chromosome 21. In our DS group, we noted a significant reduction in the NAA/Cr ratio, which is consistent with reports of other authors and may indicate an ongoing neurodegenerative process with myelinization disorders. This finding, because of a very young age of our patients, seems to confirm our previous assumption that NAA decrease appears early and is a very sensitive phenomenon preceding structural changes in the brain. We observed a tendency of Cho/Cr reduction in DS children. Choline and choline-containing compounds are considered to be the marker of degradation products of myelin that builds up the sheaths of the neural processes. The role of Cho in the development of dementia is not completely clear and there are some divergent opinions concerning its level in patients with cognitive dysfunction. Some researchers, including Kantarci et al. [28], Chantal et al.

[29], Du et al. [30] showed an age-progressing increase in the level of cholinic compounds and their higher concentrations in Alzheimer patients. We observed a tendency towards lower Cho values in DS children as compared to the control. Our data seem to be consistent with reports of such authors as Berry et al. [13], Shonk et al. [31], Huang et al. [12] and Beacher et al. [32]. The changeability of Cho concentrations suggests that these results may reflect both brain aging itself and the ongoing degenerative disease. A relatively young age of our patients as compared to elderly subjects or those with the Alzheimer-type may also play an important role. We also found lower values of the mI/Cr ratio in DS children. Myoinositol is a cyclic alcohol referred to as a glial marker. It is responsible for changes in brain osmolality and maintains normal volume of neuron. Myoinositol is associated with cell membrane metabolism, and its increase suggests gliosis. It can be found only within the astrocytes of the nervous system and is considered to be the astrocytic index and a marker of early brain damage. Shonk et al. [33] showed an increase in mI concentration in the frontal, parietal and temporal lobes, and in the temporal-parietal region in adults with DS and in Alzheimer disease. Berry et al. [13] revealed an increase in the level of mI in striatum in DS children. However, data regarding mI levels are contradictory, which has been demonstrated by Parnetti et al. [34] and Rose et al. [35]. Our findings are consistent with their reports. We found no differences in the GABA/Cr ratio between children with DS and healthy controls. GABA is the major inhibitory neurotransmitter in the central nervous system [14,36]. It is distributed within three pools in the brain. The first pool refers to the presynaptic nerve endings, the second to glial cells and the third to postsynaptic neurons. GABA synthesis takes place in the presynaptic GABA-ergic endings due to glutamate acid decarboxylation. The mechanism of GABA action is based on its reaction with specific receptors: GABA-A, GABA-B and GABA-C. In the available literature there are no reports concerning GABA concentration in ^{1}HMRS in DS patients. Our findings confirm dissociation of the stimulatory and inhibitory processes and the relationship between the content of stimulatory amino acids and disorders of maturation and "precocious aging" of the brain in DS children.

In physiological conditions, a balance exists between destruction and repair in the central nervous system. This is associated with brain plasticity observed in developmental and repair changes, as well as in the process of learning and memory. It is believed that in DS patients, destructive processes predominate over the repair ones. The apoptosis process, i.e. genetically programmed cell death, is more enhanced in patients with trisomia of chromosome 21. These data have been confirmed by our research findings [37] as well as by other authors [38,39] studying free radicals in DS patients. The elevated level of free radicals in patients with Down syndrome enhances cell destruction by, e.g. dissociation of the respiratory chain in mitochondria or destabilization of lysosomal membranes [40,41].

Proton magnetic resonance spectroscopy provides deeper insight in the chemical composition and in the ongoing metabolic processes in the brain of children with DS. It allows better understanding of pathogenesis of the disease and gives a chance of earlier and better directed rehabilitation of DS children.

Conclusions

In comparison to the control group, in the frontal lobes of children with Down syndrome MRI and ^{1}HMRS of the head revealed: 1. lack of structural changes in the brain in all DS children; 2. a statistically significant reduction in the proportion of NAA/Cr and Glx/Cr 3. decreased mI/Cr and Cho/Cr ratios; 4. unchanged GABA/Cr ratio. The findings indicate brain metabolic disorders in children with DS.

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