

AUTOANTIBODIES TO MYELIN BASIC PROTEIN AND HISTONE H1 AS IMMUNE BIOMARKERS OF NEUROPSYCHOLOGICAL DISORDERS IN PATIENTS WITH MULTIPLE SCLEROSIS

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Multiple sclerosis (MS) is a chronic autoimmune disease of the central nervous system with different disorders of neurological and higher cortical functions. It is important to identify biomarkers that can control the dynamics of neuropsychological changes and predict the progression of this process. The aim of the study was to investigate the pathogenic and clinical significance of serum autoantibodies to the myelin basic protein (MBP) and histone H1 in the occurrence of neurological and neuropsychological disorders in patients with MS. Fifty-five patients diagnosed for MS were examined. A general clinical and neurological examination, determination of cognitive status, depression level and the content of autoantibodies to histone H1 and MBP in the blood serum were conducted. Blood serum samples of 20 healthy volunteers were used as control. The serum of patients with MS was shown to contain antibodies of IgG class to MBP and histone H1. The level of anti-histone H1 IgG-antibodies in blood serum of MS patients was found to be higher compared with the level of anti-MBP IgG-antibodies ($P < 0.05$). Increased levels of anti-MBP antibodies correlated with the severity of trunk ataxia, impaired conceptualization, and mood. High level of anti-histone H1 antibodies correlated with the severity of paresis, trunk ataxia, impaired conceptualization, semantic language, and mood. The level of anti-histone H1 antibodies in blood serum of MS patients might serve as a biomarker of inflammatory and, probably, of the neurodegenerative processes of this disease and determine the dynamics of clinical course of the MS. Anti-MBP antibodies play an important role in the pathogenesis of the MS and are an additional marker of the severity of the clinical course of neurological and some neuropsychological disorders.

Key words: multiple sclerosis, IgG-autoantibodies, histone H1, myelin basic protein, cognitive impairments, depression.

Multiple sclerosis (MS) is a chronic progressive autoimmune disease of the central nervous system that mainly affects young people of working age and is one of the main causes of their disability [1, 2]. An important role in the pathogenesis of MS belongs to the processes of demyelination and neurodegeneration, so specific laboratory biomarkers are used to diagnose, determine activity and predict the course of the disease [3].

At MS, in addition to motor, sensory, cerebellar and other focal neurological disorders, changes

in higher cortical functions are observed, which in some cases may be the first and/or dominant symptom of this disease [4-6]. Neuropsychological disorders are common in MS (43 to 70%) [5, 7, 8], occur at different stages of the disease and affect the working capacity, quality of life, adaptation to chronic disease, the process of rehabilitation of patients with MS. There are impairments of memory, efficiency of information processing, executive functions, speed of information processing, attention, cognitive flexibility, impaired speech, some problems with agnosia and

apraxia, deficiency in abstract possibilities [5, 7, 8]. Various depressive manifestations were revealed [9].

At present, the mechanisms of development of neuropsychological disorders at MS remain poorly understood. One of the strategic directions of studies is to identify biomarkers that are able of controlling the dynamics of changes in neuropsychological indicators and to predict the progression of the process. Since immune pathways play an important role in the pathogenesis of MS, laboratory biomarkers have been of particular interest [3].

In patients with the MS, autoantibodies to some myelin proteins, in particular, to the myelin basic protein (anti-MBP Abs), have been detected [10, 11]. These autoantibodies are involved in the processes of demyelination [10, 11]. Their detection is used to diagnose this disease and evaluate its severity [10, 11]. The role of autoantibodies to histone H1 (anti-histone H1 Abs) in the pathogenesis of MS has been studying recently [12-14]. The functions of histone H1 are not limited to its involvement in the structural reorganization of the chromatin of the nucleus of eukaryotic cells, this protein is also present outside the nucleus and extracellularly [14-16].

It was shown that extracellular histone H1 released from damaged neurons *in vitro* activates microglial cells producing cytokines and other pro-inflammatory factors and, by causing inflammatory processes, participate in neuronal destruction [17, 18]. So, histone 1 shows its neurotoxicity in such a way [17]. It was supposed that histone H1 is involved in neuronal apoptosis, and is known to act as an autoantigen [17]. Anti-histone Abs, triggering the activation of the complement system, are involved in the mechanisms of inflammatory processes [19]. Earlier, we revealed that the level of anti-histone H1 Abs in the serum of patients with MS increases with the severity of neurological deficiency, cognitive impairments, depression, and fatigue [12, 20]. Thus, the presence of anti-histone H1 Abs in blood serum of patients with MS represents the autoimmune processes involved in the development of the disease. Their detection can be used as a biomarker of inflammatory and, probably, neurodegenerative processes, as well as a diagnostic biomarker for clinical course of the MS [12, 20].

The relevance of this work is essential for further studies of the diagnostic and prognostic role of autoantibodies to histone H1 and MBP in MS depending on clinical features of the disease and on the neuropsychological status of patients, as one of the determinants in clinical picture of the MS.

The aim of the study is to investigate the pathogenetic and clinical significance of blood serum autoantibodies to MBP and histone H1 in the occurrence of neurological and neuropsychological disorders in patients with the MS.

Materials and Methods

Between 2012 and 2015, 55 patients were examined with a reliable diagnosis of MS according to the criteria of Mac Donald 2010 at the Department of Nervous Diseases of the Danylo Halytsky Lviv National Medical University and the Lviv Regional Scientific Center for the Study of Multiple Sclerosis and other demyelinating diseases located at the Lviv Regional Clinical Hospital. All patients gave a written informed consent. The studies were conducted in accordance with the Declaration of Helsinki. Ethics approval was given by the local Ethics Committee on human research. All experimental studies were conducted in accordance with the rules of the National Congress on Bioethics (Kyiv, 2000) and the experimental protocol was approved by the Committee of Ethics in Danylo Halytsky Lviv National Medical University (protocol No 2 of February 18, 2013).

Among 55 patients, 67.27% were women, and 32.73% - men. The mean age of the patients was 38.16 ± 1.48 y.o., the mean duration of the disease was 8.29 ± 1.10 y.o., and the mean score on the Expanded Disability Status Scale (EDSS) – 4.48 ± 0.19 . Patients with recurrent-relapsing MS were predominant among the examined persons (58.18%, $n = 32$), with secondary-progressive course of MS were 25.45% ($n = 14$) patients, with primary-progressive course of MS – 7.27% ($n = 4$) and in the debut of the disease – 9.09% ($n = 5$).

All patients underwent a general clinical, neurological examination and were evaluated for cognitive status and depression. The severity of patients' neurological deficit was determined using the EDSS scale and Functional System scale (FS). A Paced Auditory Serial Addition Test, PASAT (Rao S. M., 1989), was used to evaluate the auditory information processing speed and flexibility. The Frontal Assessment Battery, FAB (Dubois B., 1999) was applied to assess executive cognitive functions (ability to conceptualize, dynamic praxis, simple and complex choice reaction, speech fluency). Phonological and semantic categories of speech fluency were investigated separately. Visual memory was examined by using the method of F. E. Rybakov (1910) and ver-

bal memory - by learning 10 words (Luria A. R., 1962). Attention indicator was evaluated according to Schulte tables (Rubinstein S. Y., 2006). The Beck Depression Scale (Beck A.T., 1961) was used to detect the depressive symptoms.

To study the level of anti-MBP IgG-antibodies and anti-histone H1 IgG – antibodies, total antibodies fractions (Abs), obtained from blood serum samples after centrifugation for 20 min at 2,000 g were used. To do this, 0.5 ml of saturated ammonium sulfate solution was added dropwise to 0.5 ml of blood serum and Ab was precipitated by centrifugation at 10,000 g for 5 min. The precipitate was dissolved in 0.5 ml of distilled water and the Ig precipitated again with 50% ammonium sulphate. After re-precipitation for three times, the Abs fraction was dialyzed for 18 h against phosphate solution (PBS) (0.01 M Na₂HPO₄, 0.01 M NaH₂PO₄, 0.145 M NaCl, pH 7.4). Measurement of the level of anti-histone H1 and anti-MBP IgG -Abs in Abs fractions was performed by means of the immuno-enzyme analysis (IEA), as described by Chamczuk et al. [21]. To do that, 96-well plates (Nunc, USA) were absorbed overnight at 4°C with human histone H1 and human MBP (8 µg/ml) in 0.1 M carbonate / bicarbonate buffer, pH 9.6. The plates were washed three times with Tris-salt buffer (TSB) containing 0.05% Tween-20, and blocked with 5% bovine serum albumin (Sigma, USA) in the same buffer for 2 h at 37°C. Abs (5 µg/ml) were added to the wells and incubated for 2 h at 37°C in the TSB containing 2% bovine serum albumin and 0.05% Tween-20. After incubation, the wells were washed three times with the TSB containing 0.05% tween-20. As “secondary” Abs, goat Abs monospecific for human heavy IgG chains conjugated to horseradish peroxidase (Jackson Labor, Germany) were used. Immuno-conjugates were detected with a solution of substrate containing: 0.1% TMB (3,3',5,5'-tetramethylbenzidine) in the DMSO (dimethyl sulfoxide) (Sigma, USA), dissolved in 100 mmol Na₂HPO₄ and 50 mmol citric acid, pH 4.5. The TMB solution and substrate buffer were mixed in a ratio of 1:10, and 0.006% hydrogen peroxide solution was added. The reaction was stopped by adding 25% sulfuric acid (1:1) to the wells. The Abs content was determined by the optical density of the solution at a wavelength of 450 nm on a scanner microplate reader BioTek (USA).

The results of patients with MS were compared with those of 20 clinically healthy people of the control group (one-time donors of the Lviv Regional Blood Transfusion Station), which included

13 (65%) women and 7 (35%) men, with an average age of 36.85 ± 3.63 years.

Statistical analysis. The results were processed using Microsoft Excel, AtteStat, SPSS and Statistica 6.0. Data was tested for normality and homogeneity of variance by using Kolmogorov-Smirnoff and Levene's tests, respectively. The arithmetic mean and standard deviation were calculated. The degree of probability was determined using the Student's *t*-test, the Mann-Whitney and Wilcoxon criteria. The Student's and xi-square criteria were calculated to compare the relative values. The Pearson's correlation test and the Spearman's rank correlation coefficient were used to assess correlations between the studied traits. Differences were considered significant when the probability was < 0.05.

Results and Discussion

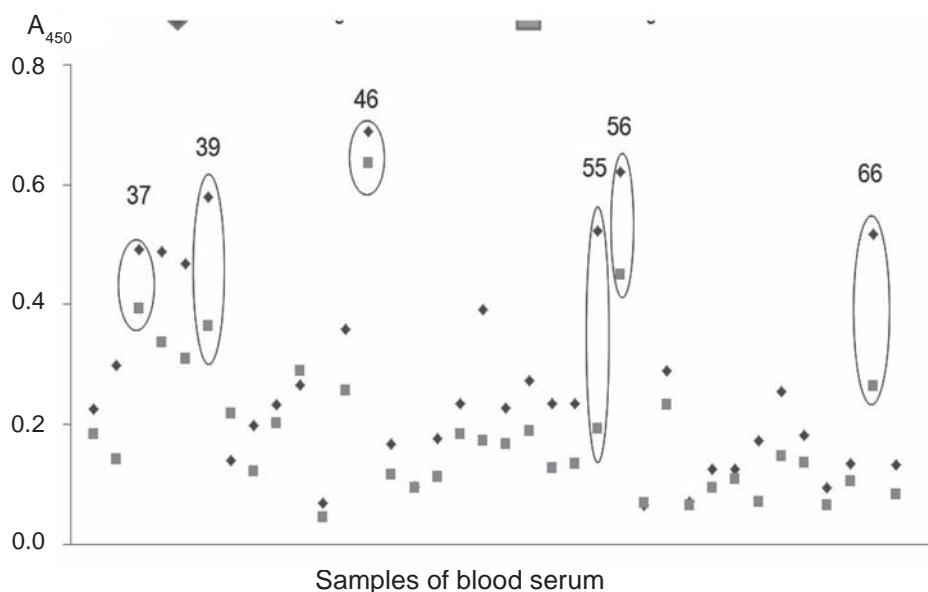
Previously, we used the immuno-enzyme analysis to investigate the presence of the anti-MBP and anti-histone H1 Abs in blood serum of patients with the MS and healthy donors [12]. The data of immuno-enzyme analysis of 36 of 55 serum samples of patients examined for IgG specific to MBP and histone H1 are shown in Figure.

It was found that IgG Abs against MBP and histone H1 are present in blood serum of patients with MS. In most serum samples of patients with MS, the level of anti-histone H1 Abs was higher than the level of anti-MBP Abs. In some samples, the difference between the levels of these antibodies is significant.

The indicators of the levels of anti-MBP and anti-histone H1 Abs in blood serum of MS patients expressed in the relative units based on optical density at A₄₅₀ are shown in Table 1.

As discovered, the mean level of anti-MBP and anti-histone H1 Abs was significantly higher in blood serum of patients with MS compared with control group ($P < 0.01$). In most samples, the level of anti-histone H1 IgG-Abs was higher than the level of IgG-Abs for the MBP, both in blood serum of patients with MS and in control group ($P < 0.05$). A positive correlation was established between the levels of these Abs ($r = 0.89$, $P < 0.05$), (Table 2). The obtained results demonstrated that with the increase of the level of one Ab, the level of the other Ab also increased.

According to the results of our previous studies, anti-MBP and anti-histone H1 Abs can serve as diagnostic markers of the severity of the patho-



Content (relative units calculated as units of optical density at A_{450}) of IgG antibodies in blood serum of patients with multiple sclerosis to myelin basic protein and histone H1 determined by the immuno-enzyme analysis (♦ – anti-histone H1 IgG antibodies; ■ – anti-MBP IgG antibodies). Serum samples of patients with MS and the highest level of anti-histone H1 IgG antibodies and the largest difference between the levels of anti-histone H1 IgG antibodies and anti-MBP IgG antibodies are circled and marked with numbers of patients

Table 1. Indicators of the level of anti-MBP and anti-histone H1 antibodies in blood serum of patients with multiple sclerosis and in control group

Studied indicator	Control group (n = 20)	Patients with MS (n = 55)
Anti-MBP Abs, relative units based on optical density at A_{450}	0.04 ± 0.01	$0.26 \pm 0.02^*$
Anti-histone H1 Abs, relative units based on optical density at A_{450}	$0.07 \pm 0.01^\#$	$0.32 \pm 0.02^{*,\#}$

Notes: * significant difference of the levels of anti-histone H1 and anti-MBP Abs between the studied and control groups, $P < 0.01$; #significant difference between the anti-histone H1 and anti-MBP Abs levels in the studied and control group, $P < 0.05$.

logical process in MS patients and determine clinical characteristics of the disease [12]. The pathogenic and clinical significance of blood serum autoantibodies to MBP and histone H1 at the neurological deficit, cognitive impairment and depressive symptoms in patients with MS were also determined. The correlation between the neurological and neuropsychological disorders in MS patients and the level of anti-histone H1 Abs and anti-MBP Abs was studied through using correlation analysis (Table 2). It has been suggested that at higher level of the investigated Abs in the blood serum of the examined patients supposes a more active course of the pathological inflammatory and neurodegenerative processes.

It was found that the increase in the level of anti-MBP Abs was less often accompanied by the pyramidal monoparesis ($r = -0.42$) and urgent calls for urination ($r = -0.31$) with a reverse correlation ($P < 0.05$). The pyramidal monoparesis and urgent calls for urination were revealed mainly in patients with mild neurological deficiency whose activity of the pathological processes was lower. That was confirmed by the detected reverse correlation between the level of the Abs to MBP and those neurological disorders. Often, an increase in the level of anti-MBP Abs was followed by the ataxia of the trunk (direct link, $r = 0.31$, $P < 0.05$). So, the severity of the neurological deficits, namely cerebellar ataxia in

Table 2. Correlation between the levels of anti-MBP and anti-histone H1 antibodies in the blood serum and clinical and neuropsychological parameters in patients with multiple sclerosis

Studied indicator	Level of Abs (relative units based on optical density at A ₄₅₀)	
	anti-histone H1	anti-MBP
Level of anti-MBP Abs, relative units based on optical density at A ₄₅₀	0.89*	1.00*
Level of anti-histone H1 Abs, relative units based on optical density at A ₄₅₀	1.0*	0.89*
EDSS, points	0.19	0.11
Visual functions (FS), points	0.15	0.24
Brainstem (FS), points	0.28	0.17
Pyramidal system (FS), points	0.34*	0.25
Cerebellar functions (FS), points	0.20	0.10
Sensory functions (FS), points	0.00	-0.08
Bowel and bladder functions (FS), points	0.09	0.01
Cerebral functions (FS), points	0.19	0.06
Pyramidal monoparesis, proportion of patients (20%; n = 11)	-0.40*	-0.42*
Ataxia of the trunk, proportion of patients (45.45%; n = 25)	0.32*	0.30*
Urgent calls for urination, proportion of patients (29.1%; n = 16)	-0.30*	-0.31*
PASAT 3, points	-0.08	-0.08
Short-term memory, word count	-0.13	-0.06
Long-term memory, word count	-0.11	-0.03
Visual memory, points	-0.15	-0.10
Impaired attention, proportion of patients (50.9%; n = 28)	0.19	0.10
FAB, points	-0.23	-0.22
Impaired conceptualization ability, proportion of patients (60%; n = 33)	0.31*	0.34*
Semantic language fluency, word count	-0.32*	-0.27
Beck's scale, points	0.09	0.03
Mood disturbance, proportion of patients (58.2%; n = 32)	0.40*	0.42*

Note: * $P < 0.05$

patients with MS, is directly related to the increase in content of blood serum anti-MBP Abs.

Correlation analysis (Table 2) revealed that high levels of anti-MBP Abs in blood serum of MS patients were accompanied by the impaired conceptualization ($r = 0.34$) and higher proportion of patients with mood disorders ($r = 0.42$, $P < 0.05$).

Thus, an increase in the level of anti-MBP Abs in blood serum of MS patients correlates with the severity of trunk ataxia, disorders of conceptualization and mood. The obtained data showed the connection between an increase in the levels of anti-MBP Abs in blood serum and the severity of neurological deficits and some neuropsychological disorders in

MS patients. This is consistent with the results of other investigators that the determination of blood serum anti-MBP Abs can be used for evaluation of the severity of MS [10-12]. So, anti-MBP Abs play an important role in the pathogenic mechanisms of the development of neurological manifestations, cognitive impairments and depressive symptoms in MS patients and can serve as a diagnostic marker of clinical course of this disease.

Changes in the level of anti-histone H1 Abs in blood serum depending on the neurological deficiency, cognitive disorders and depressive symptoms of MS patients were studied using correlation analysis (Table 2). A direct correlation between the

degree of impairment of the pyramidal system on the EDSS scale and an increase in the level of anti-histone H1 Abs in blood serum of patients with MS was revealed ($r = 0.34$, $P < 0.05$). At the same time, pyramidal monoparesis ($r = -0.40$) and urgent calls to urination ($r = -0.30$) were observed less frequently ($P < 0.05$). As in the case of anti-MBP Abs, these results indicate that monoparesis and urgent calls for urinations were symptoms of easily expressed neurological deficit in MS patients whose activity of pathological processes was lower. As the level of anti-histone H1 Abs increased, ataxia of the trunk was more common (direct link, $r = 0.32$, $P < 0.05$). Thus, the severity of neurological deficit, namely central paresis and cerebellar ataxia in MS patients, is directly related to an increased content of anti-histone H1 Abs in blood serum.

At determining the correlation between indicators of patients' cognitive function and blood serum anti-histone H1 Abs, a correlation was found between disorders of ability to conceptualize, speech fluency and levels of these Abs (Table 2). A direct correlation was revealed between the proportion of people with impaired conceptualization ($r = 0.31$), and an reverse correlation - between the semantic speech fluency indicator ($r = -0.32$) and the level of Abs ($P < 0.05$). Assuming that the activity of the pathological process in MS is associated with the level of anti-histone H1 Abs in blood serum of MS patients, we might conclude that the deterioration of the semantic category of speech fluency and thinking occurs with increasing activity of the pathological process.

Examination of the relation of depressive manifestations on the Beck scale in MS patients and the level of anti-histone H1 Abs in blood serum (Table 2) revealed that with an increase in the level of these Abs, a higher proportion of MS patients with mood disorders was observed ($r = 0.40$, $P < 0.05$).

So, worse indicators of neurological deficiency, cognitive impairments, depressive manifestations were found at a higher level of anti-histone H1 Abs in blood serum of MS patients. High level of anti-histone H1 Abs correlated with more significant disorders in the pyramidal system, trunk ataxia, impaired conceptualization, semantic speech fluency and mood.

The role of anti-histone H1 Abs at the diseases of nervous system is discussed not only as a marker of inflammation, but also of neurodegeneration [12, 17, 19]. A positive correlation between the level of

anti-histone H1 Abs in blood serum and the indicators of neurological deficiency and neuropsychological disorders of the examined people confirmed this assumption. The results of this study indicate that anti-histone H1 Abs, as well as anti-MBP Abs, play an important role in the pathogenesis of MS and can serve as a diagnostic and prognostic marker of clinical course of the neurological and cognitive disorders and depressive manifestations.

Conclusions. IgG antibodies specific for MBP and histone H1 are present in blood serum of MS patients ($P < 0.01$, compared with healthy donors). The level of anti-histone H1 IgG-Abs was significantly higher than the level of anti-MBP IgG-Abs in the same Abs fractions isolated from blood serum of MS patients. High levels of anti-histone H1 and anti-MBP Abs in blood serum of MS patients correlated with the severity of trunk ataxia, disorders of conceptualization and mood ($P < 0.05$). There was a higher dependence of the neurological deficit and cognitive impairments upon the level of anti-histone Abs than upon the anti-MBP Abs. As their level increased, more significant disorders of the pyramidal system and the semantic speech fluency category were observed ($P < 0.05$). Thus, determination of the level of the anti-histone H1 Abs in blood serum of patients with MS may serve as the biomarker of not only inflammatory and neurodegenerative processes of this disease, but also might determine the dynamics of its clinical course. Anti-MBP Abs also play an important role in pathogenesis of the MS and are an additional marker of the severity of clinical course of neurological and specific neuropsychological disorders.

Further studies are necessary to clarify the role of the IgG antibodies to histone H1 and to MBP in pathogenesis of the MS, as diagnostic and prognostic markers of the neurological and neuropsychological disorders.

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АВТОАНТИТІЛА ДО ОСНОВНОГО ПРОТЕЇНУ МІЄЛІНУ ТА ГІСТОНУ Н1 ЯК ІМУННІ БІОМАРКЕРИ НЕЙРОПСИХОЛОГІЧНИХ РОЗЛАДІВ У ХВОРИХ НА РОЗСІЯНИЙ СКЛЕРОЗ

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Розсіяний склероз (РС) – хронічне демієлінізуюче аутоімунне захворювання центральної нервової системи, за якого спостерігаються вогнищеві неврологічні порушення та розлади вищих кіркових функцій. Важливим є виявлення біомаркерів, які здатні контролювати динаміку змін нейропсихологічних показників, передбачати прогресування цього процесу. Мета роботи – дослідити патогенетичне і клінічне значення аутоантитіл сироватки крові до основного протеїну мієліну (ОПМ) і гістону Н1 у виникненні неврологічних та нейропсихологічних розладів у хворих на РС. Обстежено 55 пацієнтів із РС, яким проводили загальноклінічне, неврологічне дослідження, оцінювали когнітивний статус, рівень депресії та визначали вміст аутоантитіл до гістону Н1 та ОПМ у сироватці крові за допомогою імунологічного методу. Результати хворих на РС порівнювали з показниками 20 практично здорових осіб. У сироватці крові пацієнтів із РС присутні антитіла (АТ) класу IgG до ОПМ та гістону Н1. Було показано, що сироватка пацієнтів з РС містила антитіла класу IgG до гістону Н1 та ОПМ. Показано, що спорідненість антитіл до гістону вища

порівняно з їх спорідненістю до ОПМ ($P < 0,05$). Високий рівень антигістонових антитіл до Н1 корелював із тяжкістю парезу, атаксією стовбура, порушенням концептуалізації, семантичною мовою та настроєм. Підвищений рівень антитіл до ОПМ корелював з тяжкістю атаксії тулуба, порушенням концептуалізації та настроєм. Рівні антигістонових Н1 та анти-ОПМ антитіл у сироватці крові хворих на РС можуть служити біомаркерами неврологічних розладів та динаміки їх клінічного перебігу.

Ключові слова: розсіяний склероз, IgG-аутоантитіла, гістон Н1, основний протеїн мієліну, когнітивні порушення, депресія.

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