NIEMANN-PICK TYPE C DISEASE AS AN EXAMPLE OF NEURODEGENERATIVE LYSOSOMAL STORAGE DISORDER

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Lysosomes are one of the key components of all eukaryotic cells. They are made as spherical vesicles that contain hydrolytic enzymes for breaking down biomolecules inside the cell. During second half of twentieth century and in the beginning of the new age lysosomes have been continuing to attract attention of cell biologists all over the world. Scientists' efforts have lead to establishment of cell autophagy phenomenon – typical intracellular process based on lysosomal self-degradation, which plays a critical role in maintaining cell homeostasis [5]. This process was discovered and studied by Yoshinori Ohsumi and his group. He received the 2016 Nobel Prize in Physiology or Medicine for his discoveries of mechanisms for autophagy [18].

Lysosomal storage diseases (LSDs) are severe conditions that occur as a result of impairment of genes coding lysosomal enzymes or some other proteins crucial for lysosome functioning. Fifty different subtypes of LSDs are described according to types of metabolites – lipid storage disorders (first of all, glycosphingolypidoses), mucopolysaccharidoses, glycoprotein storage disorders, mucolipidoses, neuronal ceroid lipofuscinoses, etc. [6]. So-called glycogen storage disease type II (Pompe disease) was described first in the LSDs group. The deficit of acid α-1, 4-glucosidase primarily in muscles and myocardium underlies the Pompe disease. All LSDs belong to orphan diseases.

Genetics and pathophysiology

Niemann-Pick type C disease refers to glycosphingolypidoses but it has different pathophysiology relative to other forms. It affects all age groups, and the prevalence is the highest among children and young adults. It steadily progresses and leads to severe disability and early death. Not an acid lysosomal hydrolase deficite but abnormal intracellular cholesterol transport results in unetherefied cholesterol fraction, sphyngomyeline and other glycosphyngolypides accumulation in the central nervous system, reticuloendothelial system (liver and spleen) and lungs [2,22,26]. NPC is named after German doctor Albert Niemann and pathologist Ludwig Pick who described the group of LSDs in the first half of the XX century. In 1961 A.Crocker divided Niemann-Pick disease into four types A, B, C, D in respect of variable age of onset and clinical picture [11]. And now term Niemann-Pick disease includes two

essentially different groups of LSDs – with deficit of acid sphingomyelinase caused by *SMPD1* gene mutation in 11p15.4-p15.1 locus (types A and B) and type C with endocellular cholesterol transport impairment. NPC is caused by *NPC1* (locus 18q11.2) or *NPC2* (locus 14q24.3) gene mutations. Type D, described in patients originated from New Scotland, part of Canada, is thought of as a subtype of NPC.

In NPC primary molecular defect has been identified in two separate genes responsible for development of the same pathological phenotype [9,20]. NPC1 gene mutation occurs in 95% of families. NPC2 gene mutation is less frequent (4% cases), and 1% of cases assume mutations that are not yet identified. NPC1 gene product is an integrated membrane protein localized in the “late” endosomes; this protein is considered to be critically important for modulation of cholesterol and glycosphingolipid redistribution. NPC2 gene encodes soluble cholesterol transporter. Both proteins work closely with each other coordinating intracellular sterol metabolism indirectly via oxysterols [14, 25, 27]. Gene mutations mentioned above lead to cholesterol etherification impairment, abnormal intracellular cholesterol transport and apoptotic cascade activation, lysosomal storage of big amount of different lipids, secondary sphyngolipid metabolism disfunction with cell death in target organs [16,19,22,24]. NPC is lysosomal neurodegenerative disorder, which affects virtually all parts of the central nervous system. NPC refers to the group of orphan diseases – minimally estimated prevalence is limited to 1:120000 liveborn infants [29], the prevalence in population is estimated as 1:1,25 million. There are reasonable grounds to believe that the prevalence of NPC in population is underestimated because of significant underdiagnosis.

Classification, clinical features and diagnosis

There are neonatal, infantile (early and late), juvenile and adult forms of NPC according to the age of onset of the disease [29]. Juvenile onset is most common (60% of all cases), first signs develop at 6 to 12 years old. The NPC is characterized by visceral involvement (liver, spleen, and sometimes lung), neurologic and psychiatric manifestations [17] (table). Visceral symptoms are most prominent in neonatal and early infantile onset forms. Neurologic and psychiatric symptoms prevail with increase in age. Most severe cases include neonatal and early infantile onsets of NPC, characterized by fetal ascites, prolonged jaundice or neonatal cholestasis, hepatosplenomegaly, pulmonal infiltrates, seizures, diffuse muscle hypotonia. In late infant form there is hepatosplenomegaly or isolated splenomegaly, revealed on ultrasound sonography, psychomotor and speech retardation, progressive neurologic signs in children of 2-6 years old. 30-

50% of patients develop partial or generalized seizures, half of affected children reveal gelastic cataplexy – paroxysmal generalized muscle atonia that lead to falls without conscious loss, usually provoked by positive emotional reactions. Typical primary features are clumsiness and progressive decrease in school performance. Later on motor disturbances and ataxia develop, then focal or generalized dystonias occur, choreoathetoid hyperkinetic movements, myoclonias, thremor, bradikynesia, motor and spatial apraxia may develop, bulbar and pseudobulbar paralysis appear, progredient cognitive decline is usually seen. Vertical supranuclear gaze palsy (VSGP) is almost invariably present and often the initial sign in 90% cases. Classic motor triade is always suspicious for NPC – VSGP with ataxia and dystonia in young adults [17]. Adolescent and adult neurologic onset after 12 years old is characterized by slow progression until the age 50-60 years old. Specific feature of adult onset form is the presence of psychiatric symptoms including affective disorders, obsessive-compulsive disorder, and schizophrenia-like disorder with deliriation, hallucinations, paranoid delusions, and aggressive behavior. Spectrum of neurologic symptoms is almost the same in juvenile and adult onset forms.

Table. Classification of signs and symptoms in NPC.

Visceral	
	Isolated unexplained splenomegaly
	Hepatomegaly/Splenomegaly
	Prolonged neonatal cholestatic jaundice
	Hydrops foetalis or foetal ascites
	Pneumopathologies (aspiration pneumonia, alveolar lipidosis, interstitial lung involvement)
	Mild thrombocytopenia
Neurological	
	Vertical supranuclear gaze palsy
	Gelastic cataplexy
	Ataxia
	Dystonia
	Dysarthria
	Dysphagia
	Hypotonia

	Clumsiness
	Delayed developmental milestones
	Seizures
	Hearing loss
Psychiatric	
	Developmental delay and pre-senile cognitive decline
	Organic psychosis
	Disruptive/aggressive behavior
	Progressive development of treatment-resistant psychiatric symptoms

Clinical evaluation allows to reveal key symptoms, perform differential diagnosis for exclusion of other possible causes of neurodegenerative and visceral symptoms. The Suspicion Index tool developed by scientific expert group [1, 4, 21, 30] is a screening tool that can help identify patients who may warrant further investigation for NP-C. Data on levels of cholesterol oxidation products (oxysterols) in animals and humans with NPC1 mutations have recently been reported, indicating that they are sensitive and specific markers for NP-C screening [7, 8, 15, 21]. Direct sequencing of all NPC1 and NPC2 exons is a gold standard of NPC diagnostic methods [21]. Molecular-genetic testing is essential for evaluating subjects from risk group in affected family. It is performed only after gene mutation has been identified among family members. Healthy gene carriers are heterozygotes and have no risk of disease in the future. DNA analysis is usually performed as prenatal diagnosis in women from risk group aiming family planning [1].

Substrate reduction therapy, own experience

Pathogenetic therapy has been developed for NPC. There is only one approved medication for treatment of neurologic symptoms in children and adults, it is called miglustat and designed for life-long intake as a substrate reduction therapy. The other indication for miglustat is Gaushe disease type I. Miglustat has been registered in Russia since 2009 with the brand name Zaveska. It is an N-alkylated iminosugar that inhibits glycosilceramide sintase catalyzing first stage synthesis of glycosphingolipids that leads to less intracellular storage (pic.1).

To prevent the disease progression treatment with miglustat should be initiated when neurological symptoms occur [21]. If there is no neurologic symptoms miglustat is not indicated. The drug is available in capsules 100 mg, is taken orally without

regard to food in the dose 200 mg 3 times a day (adults and children older than 12 years old). Rated dose for children younger than 12 years old is calculated according to the total body area. Miglustat cross the blood-brain barrier and decreases cholesterol and glykosphingolipid storage in neuron lysosomes, thus reduces neurological symptoms progression and makes the survival rate higher according to preclinical data. It also regulates intracellular calcium homeostasis connected to sphyngosine storage that assumed as launch factor in NPC patogenesis by changing glucosilceramid concentration. Miglustat improves oculomotor functions, cognition, swallowing, decreases motor impairment, psychotic symptoms [10, 12, 13]. It doesn't affect visceral symptoms. Typical side effects include body weight loss observed in 60% of patients during first year of treatment, diarrhea and other dispeptic complaints related to concurent inhibition of dysacharidase in gastrointestinal tract.

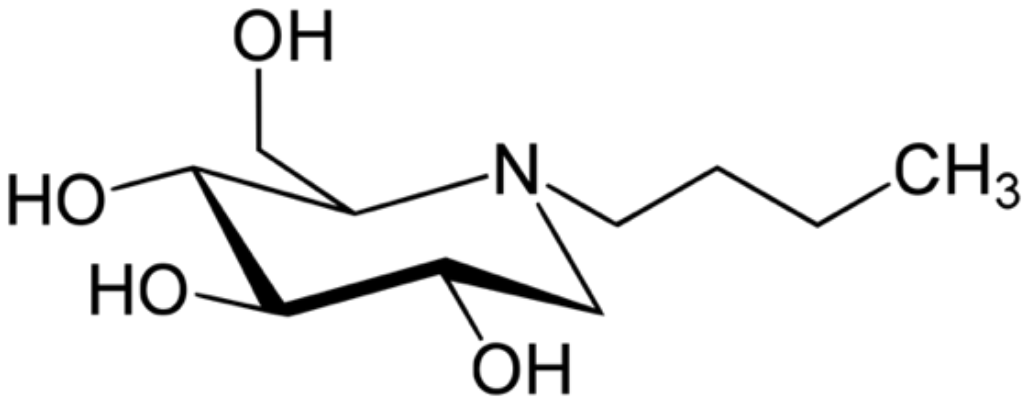

Fig.1. Miglustat (N-butyl-deoxynojirimycin).

We have two NPC patients receiving miglustat – 29-year old woman (onset at 6 years) and 21-year old man (onset at 9 years) with typical clinical features – VSNGP, dystonia, ataxia, dementia, splenomegaly, diffuse brain atrophy on MRI (pic.2). While continuous miglustat intake 600 mg a day (woman – 3,5 years, man – 3 years) we observe stabilization of disease progression, decrease in ataxia severity and other motor symptoms according to clinical scales. Increase in quality of life and social adaptation was observed. Drug tolerance is good in general. The female patient had 10-12% weigth loss during first year of therapy, and then it changed back. Patients don’t have any dyspeptic symptoms. Three more patients with juvenile onset form have started to take miglustat. Response to treatment will be measured later.

Conclusion

NPC is an outstanding example of progressive lysosomal neurodegenerative disorder with established substrate reduction therapy. We are looking forward to see the new convincing treatment options for this condition. There is study ongoing with cholesterol-binding compound, cyclodextrin (CYCLO) for the removal of unesterified cholesterol from late endosomes/lysosomes [28], and also histone deacetylase inhibitors (HDAC), which act by increasing expression of the low transport activity NPC1 mutant protein and significantly reduce neuronal death [23]. The level of modern molecular neurobiology gives hope to the development of disease-modifying therapy for other severe neurodegenerative disorders.

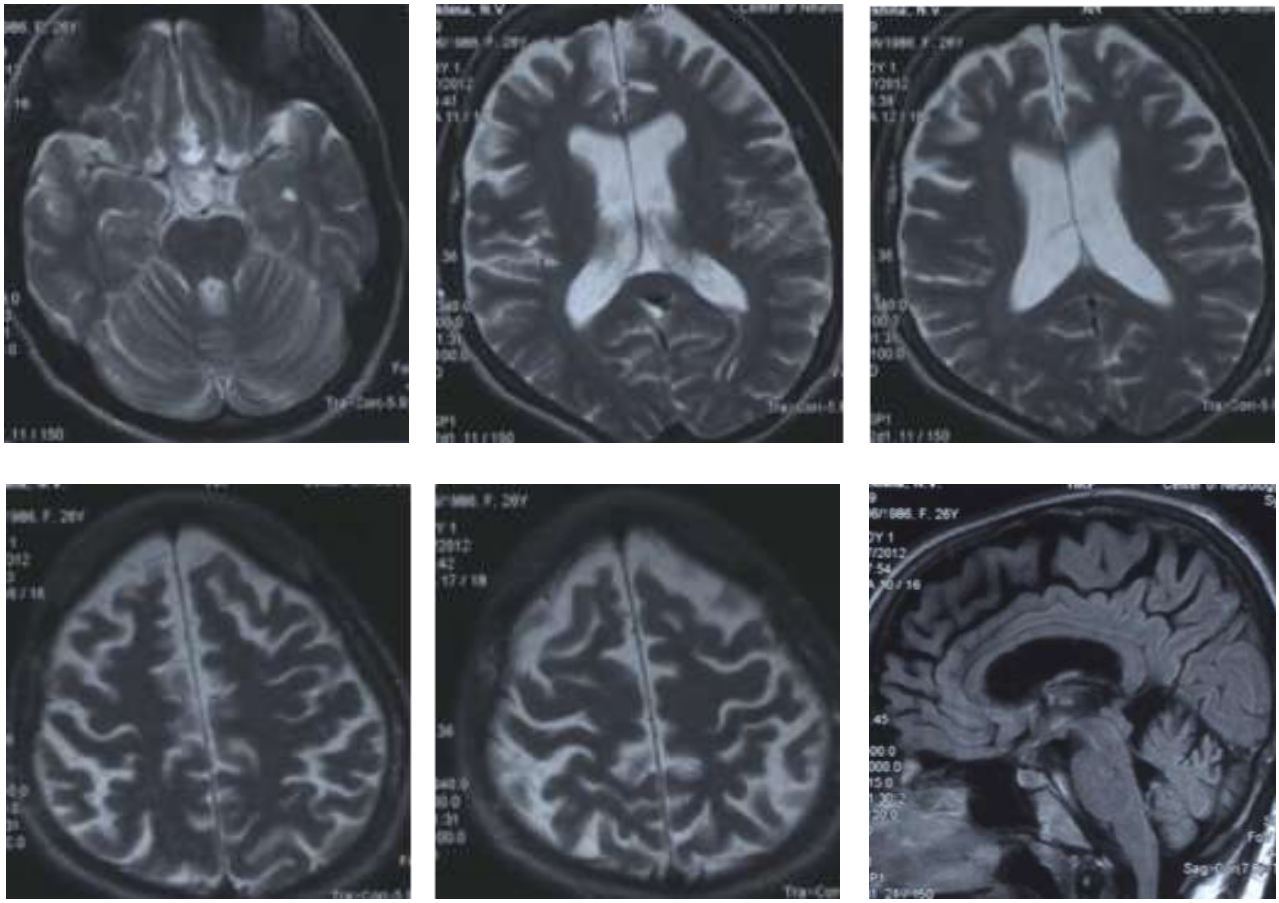

Fig.2. MRI. Diffuse brain atrophy in 21-year old NPC patient.

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