ASSESSMENT OF GENETICS MUTATIONS IN SMPD1, NPC1, NPC2 GENES TO INDUCED NIEMANN–PICK SYNDROME

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ABSTRACT

In this study we have analyzed 30 people. 10 patients Niemann–Pick disease and 20 persons control group. The genes SMPD1, NPC1, NPC2, analyzed in terms of genetic mutations made. In this study, people who have genetic mutations were targeted, with nervous disorders Niemann–Pick disease. In fact, of all people with Niemann–Pick disease.. 10 patients Niemann–Pick disease had a genetic mutations in the genes SMPD1, NPC1, NPC2 Niemann–Pick disease.Any genetic mutations in the target genes control group, did not show.

KEYWORDS: Genetic study, Niemann–Pick disease, Mutations the gene SMPD1, NPC1, NPC2, RT-PCR.

INTRODUCTION

Niemann–Pick disease (/niːmənˈpɪk/nee-mən-PIK)1 is a group of inherited, severe metabolic disorders in which sphingomyelin accumulates in lysosomes in cells. The lysosomes normally transport material through and out of the cell.

The prognosis is individual, but the severe form is fatal in toddlerhood and, in some cases, patients with the milder forms may have normal lifespans.
This disease involves dysfunctional metabolism of sphingolipids, which are fats found in cell membranes, so it is a kind of sphingolipidosis. Sphingolipidoses, in turn, are included in the larger family of lysosomal storage diseases.\textsuperscript{[2]}

**Signs and Symptoms**

Symptoms are related to the organs in which sphingomyelin accumulates. Enlargement of the liver and spleen (hepatosplenomegaly) may cause reduced appetite, abdominal distension, and pain. Enlargement of the spleen (splenomegaly) may also cause low levels of platelets in the blood (thrombocytopenia).

Accumulation of sphingomyelin in the central nervous system (including the cerebellum) results in unsteady gait (ataxia), slurring of speech (dysarthria), and difficulty in swallowing (dysphagia). Basal ganglia dysfunction causes abnormal posturing of the limbs, trunk, and face (dystonia). Upper brainstem disease results in impaired voluntary rapid eye movements (supranuclear gaze palsy). More widespread disease involving the cerebral cortex and subcortical structures causes gradual loss of intellectual abilities, causing dementia and seizures.

Bones also may be affected: symptoms may include enlarged bone marrow cavities, thinned cortical bone, or a distortion of the hip bone called coxa vara. Sleep-related disorders, such as sleep inversion, sleepiness during the day and wakefulness at night, may occur. Gelastic cataplexy, the sudden loss of muscle tone when the affected patient laughs, also is seen.

**Niemann–Pick disease** has an autosomal recessive pattern of inheritance.

Mutations in the *SMPD1* gene cause Niemann–Pick disease types A and B. They produce a deficiency in the activity of the lysosomal enzyme acid sphingomyelinase, that breaks down the lipid sphingomyelin.\textsuperscript{[3]}

Mutations in *NPC1* or *NPC2* cause Niemann–Pick disease, type C (NPC), which affects a protein used to transport lipids.\textsuperscript{[3]}

Type D originally was separated from type C to delineate a group of patients with otherwise identical disorders who shared a common Nova Scotian ancestry. Patients in this group are known to share a specific mutation in the *NPC1* gene, so NPC is used for both groups. Before the molecular defects were described, the terms "Niemann–Pick type I" and "Niemann–Pick
type II" were proposed to separate the high- and low-sphingomyelin forms of the disease in the early 1980s.

Niemann–Pick disease is inherited in an autosomal recessive pattern, which means both copies, or both alleles, of the gene must be defective to cause the disease. "Defective" means they are altered in a way that impairs their function. Most often, the parents of a child with an autosomal recessive disorder are carriers: they have one copy of the altered gene, but are not affected because the other copy produces the enzyme. If both parents are carriers, each pregnancy has a 25% chance of producing an affected child. Genetic counseling and genetic testing are recommended for families who may be carriers of Niemann–Pick.

Niemann–Pick diseases are a subgroup of lipid storage disorders called sphingolipidoses in which harmful quantities of fatty substances, or lipids, accumulate in the spleen, liver, lungs, bone marrow, and brain.

In the classic infantile type-A variant, a missense mutation causes complete deficiency of sphingomyelinase. Sphingomyelin is a component of cell membrane including the organelar membrane, so the enzyme deficiency blocks degradation of lipid, resulting in the accumulation of sphingomyelin within lysosomes in the macrophage-monocyte phagocyte lineage. Affected cells become enlarged, sometimes up to 90 μm in diameter, secondary to the distention of lysosomes with sphingomyelin and cholesterol. Histology shows lipid-laden macrophages in the marrow and "sea-blue histiocytes" on pathology. Numerous small vacuoles of relatively uniform size are created, giving the cytoplasm a foamy appearance.

**PATHOLOGY**

Loss of myelin in the central nervous system is considered to be a main pathogenic factor. Research uses animal models carrying the underlying mutation for Niemann–Pick disease, e.g. a mutation in the NPC1 gene as seen in Niemann-Pick type C disease. In this model, the expression of myelin gene regulatory factor (MRF) has been shown to be significantly decreased.[15] MRF is a transcription factor of critical importance in the development and maintenance of myelin sheaths.[16] A perturbation of oligodendrocyte maturation and the myelination process might, therefore, be an underlying mechanism of the neurological deficits.[15]
In 2011, fibroblast cells derived from patients with Niemann-Pick type C1 disease were shown to be resistant to Ebola virus because of mutations in the NPC1 protein, which is needed for viral escape from the vesicular compartment.[17]

Other studies have unturned small molecules which inhibit the receptor and may be a potential therapeutic strategy.[18]

Treatments under investigation

Experimental use of arimoclomol

In 2014 the European Medicines Agency (EMA) granted orphan drug designation to arimoclomol for the treatment of Niemann-Pick type C.[19] This was followed in 2015 by the U.S. Food & Drug Administration (FDA).[20] Dosing in a placebo-controlled phase II/III clinical trial to investigate treatment for Niemann-Pick type C (for patients with both type C1 and C2) using arimoclomol began in 2016.[21]

Experimental use of 2-hydroxypropyl-β-cyclodextrin

Researchers at the University of Texas Southwestern Medical Center found, when Niemann–Pick type C mice were injected with 2-hydroxypropyl-β-cyclodextrin (HPbCD) when they were 7 days old, marked improvement in liver function tests, much less neurodegeneration, and, ultimately, significant prolongation of life occurred. These results suggest HPbCD acutely reverses the storage defect seen in NPC.[22]

In April 2011, the U.S. National Institutes of Health (NIH), in collaboration with the Therapeutics for Rare and Neglected Diseases Program (TRND),[23] announced they are developing a clinical trial using HPbCD for Niemann–Pick type C patients. The clinical trial is in the planning phase, not yet approved by the FDA.[24]

MATERIALS AND METHODS

In this study, 10 patients with Niemann–Pick disease and 20 persons control group were studied. Peripheral blood samples from patients and parents with written permission control was prepared. After separation of serum, using Real Time-PCR technique of tRNA molecules were collected. To isolate Neuroglial cells erythrocytes were precipitated from hydroxyethyl starch (HES) was used. At this stage, HES solution in ratio of 1to5with the peripheral blood of patients and controls were mixed. After 60 minutes of incubation at room temperature, the supernatant was removed and centrifuged for 14 min at 400 Gera. The cells sediment with
PBS (phosphate buffered saline), pipetazh and slowly soluble carbohydrate ratio of 1 to 2 on ficole (Ficol) was poured in the 480G was centrifuged for 34 minutes. Mono nuclear Neuroglial cells also are included, has a lower density than ficole and soon which they are based. The remaining erythrocytes has a molecular weight greater than ficole and deposited in test tubes.

The supernatant, which contained the mono nuclear cells was removed, and the 400 Gera was centrifuged for 12 minutes. Finally, the sediment cell, the antibody and Neuroglial cells was added after 34 minutes incubation at 5 °C, the cell mixture was passed from pillar LSMACS. Then the cells were washed with PBS and attached to the column LSMACSS pam Stem cell culture medium containing the transcription genes SMPD1, NPC1, NPC2, and were kept.

To determine the purity of Neuroglial cells are extracted, flow cytometry was used. For this purpose, approximately 4-5 × 10^3 Neuroglial cells were transfer red to 1.5ml Eppendorf tube and then was centrifuged at 2000 rpm for 7 minutes at time. Remove the supernatant culture medium and there maining sediment, 100μl of PBS buffer was added. After adding 5-10μl PE monoclonal anti body to the cell suspension for 60 min at 4°C, incubated and readimme diately by flow cytometry. For example, rather than control anti body Neuroglial cells PE, IgG1 negative control solution was used.

**Total mRNA extraction proced ures cludes**

1) 1ml solution spilled Qiazolon cells, and slowly and carefully mixed and incubated at room temperature for 5 minutes. Then 200μl chloroform solution to target mix, then transfer the micro tubes were added, and the shaker well was mixed for 15 seconds. The present mix for 4 minutes at room temperature and then incubated for 20 min at 4°C on was centrifuged at 13200 rpm era. Remove the upper phase product were transfer reductase new microtube and to the one times the volume of cold ethanol was added. The resulting mixture for 24 hours at -20°C were incubated.

2) Then for 45 min at 4°C on was centrifuged at 12000 rpm era. Remove the super natant and the white precipitate, 1ml of cold 75% ethanol was added to separate the sediment from micro tubes were vortex well. The resulting mixture for 20 min at 4°C on by the time we were centrifuged 12000 rpm. Ethanol and the sediment was removed and placed at room temperature until completely dry deposition. The precipitate was dissolved in 20μl sterile water and at a later stage, the concentration of extracted mRNA was determined.
To assessment the quality of mi-RNAs, the RT-PCR technique was used. The cDNA synthesis in reverse transcription reaction (RT) kit (Fermentas K1622) and 1μl oligoprimers 18 (dT) was performed. Following the PCR reaction 2μM dNTP, 1μg cDNA, Fermentas PCR buffer 1X, 0 / 75μM MgCl2, 1.25 U / μL Tag DNA at 95°C for 4 min, 95°C for 30s, annealing temperature 58°C for 30s, and 72 °C for 30 seconds, 35 cycles were performed. Then 1.5% agarose gel, the PCR product was dumped in wells after electrophores is with ethidium bromide staining and colors were evaluated.

RESULTS

Figure 1: Schematic representation of NPC1 and NPC2 genes under the contrast phase microscope compared to the normal group.
Figure 2: Schematic representation of NPC1 and NPC2 genes The types of microbial objects under the contrast phase microscope compared with the normal group.
Figure 3: Schematic representation of band formation pattern in NPC1 and NPC2 genes compared to normal group.
Figure 4: Microscopic Image of Cell Culture in NPC1 and NPC2 Genes Compared to the Normal Group.
Figure 5: Bioinformatics diagram of the expression of NPC1 and NPC2 genes in blood and brain bone versus the normal group.
Figure 6: Bioinformatics diagram of the expression of NPC1 and NPC2 genes in the synthesis of cholesterol and Triolein Genotypes.

Figure 7: Diagram of the expression of NPC1 and NPC2 genes in blood and white blood cell lymphocytes compared to the normal group.
Figure 8: Microscopic image of the expression of NPC1 and NPC2 genes in blood lymphocytes and white blood cells compared to normal group.

Figure 9: Schematic of the formation pattern and NPC2 gene expression in blood lymphocytes and white blood cells in patients.
Figure 10: Schematic of the bond formation pattern and expression level of NPC genes in the brain cells of the affected patients.
Figure 11: Schematic of the pattern of bond formation and expression of NPC genes in brain cells in patients with brain tumors.
Figure 12: Schematic of the formation pattern of the genes in the mutated NPC genes compared to the normal group.

Figure 13: Schematic of the bond formation pattern in the mutated NPC genes after incubation in hours in the cytoplasmic cell line.
Figure 14: Schematic of bond formation pattern in NPC mutated genes after incubation with DMSO.
DISCUSSION AND CONCLUSION

According to the results of sequencing the genome of patients with Niemann–Pick disease, and the genetic mutations SMPD1, NPC1, NPC2 genes found that about 100% of patients with Niemann–Pick disease, they have this genetic mutations. Patients with Niemann–Pick disease, unusual and frightening images in the process of Niemann–Pick disease, experience. Lot epigenetic factors involved in Niemann–Pick disease. But the most prominent factor to induce Niemann–Pick disease, mutations is SMPD1,NPC1,NPC2 genes. This genes can induce the birth and can also be induced in the adulthood.
Figure 16: A schematic map of the frequency of Niemann–Pick disease outbreaks in the world.

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