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Original Research Article

A review of the promising new advances in the combat against cholesterol trafficking defect and consequent occurrence of the rare Niemann-Pick type C disease

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Abstract

Introduction: The rare, often neglected, and incurable Niemann-Pick type C (NPC) disease is alysosomal storage disorder that occurs in many communities across the world, affecting largely youngsters. The slow neurodegeneration caused by this disease is the primary and eventual cause of death for NPC patients in most cases. Since NPC still remains incurable, extensive focus and considerable efforts have been made by biomedical researchers in order to combat its many intricacies including but not limited to lipid homeostasis that leads to neurological consequences for NPC patients.

Objectives and Method: This review summarizes recent and most relevant studies and triages the important aspects of NPC which need to be addressed and are of immediate concern to the scientific community. Extensive literature review was conducted using the PubMed database and biomedical literature from MEDLINE to identify the most recent and relevant research and emphasis was put on identifying promising new ways by which NPC and cholesterol trafficking defect may be combated.

Results and Conclusions: Many neuronal and non-neuronal studies have been carriedouton lipid trafficking alterations to advance the knowledge of NPC. It has been observed that β-cyclodextrin therapy, substrate reduction therapy with Miglustat, and histone deacetylase (HDAC) inhibitors have proven to be the most promising therapeutic agents in this regard but other treatment options are also available as revealed by literature review. However, further studies are warranted in order to identify or lessen the nebulous correlation between lipid trafficking defects and the clinical manifestations of NPC. Considering the difficulty in NPC diagnosis and effective treatment for NPC, it is imperative for researchers to well aware of potential therapeutic targets, agents and strategies that might be useful in the near future. A better understanding of NPC and its evaluation on potential treatment options would have a significant effect on the therapy and management of NPC patients.

Keywords: Niemann-Pick type C, Cholesterol trafficking defect, Neurodegenerative disease, Lipid homeostasis, Lysosomal Storage Disorder.

1. Introduction

A relatively rare and currently incurable neurovisceral disease, Niemann-Pick type C (NPC) is estimated to prevail at a rate of 1 in 150,000 individuals around the world[1]. Those who are affected by this disease usually die before they reach adulthood but the progression can be slower for those whose age of onset is at a later part of life. NPC is very different from Niemann-Pick Type A and B from a clinical standpoint and is characterized as autosomal recessive.

NPC is usually caused by specific genetic mutations, in particular, the mutation in the *npc1* gene on chromosome 18 accounts for 95% of the cases [2-4]. On the other hand, about 5% of the cases of NPC can be attributed to a mutation in the gene, *npc2* on chromosome 14 [5-7]. The characteristics of this disease usually include neurodegeneration of the central nervous system and progressive hepatosplenomegaly. Under NPC diseased condition, cholesterol i.e. un-esterified cholesterol and other lipids may accumulate within the cells of various tissues and also the brain. Even though there is no cure for NPC, as of yet, extensive biochemical studies have shown promise with regards to the slowing of disease progression [1, 8-10].

1.1. A biochemical overview of NPC Proteins:

The *npc1* gene encodes a glycoprotein NPC1, consisting of 1252 amino acids, that includes a "sterol sensing domain" or SSD on residues 615 to 797homologies with sterol regulatory element-binding protein cleavage-activating protein (SACP) and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase). This domain is located between the 3rd and 7th transmembrane domains out of thirteen transmembrane domains of this glycoprotein. This 180 amino acid domain is organized in five transmembrane domains itself which is found on membrane proteins that are involved in various cellular processes such as: cell-cell signaling, cholesterol homeostasis and dietary cholesterol uptake [1,2,11-16]. These SSDs function to mediate sterol binding and are necessary for NPC1 proteins to function inside the intact cells. A binding process takes place between NPC1 protein and an analog of cholesterol known as azocholestanol. This binding process is blocked in part by cholesterol. For NPC1 proteins that contain mutations within the SSD, the extent of this binding is diminished further [7, 17-19]. NPC1 may also act as a lipid permease, but the role of SSD in mediating the activity of permease has not been determined yet. The primary location of NPC1 is the late endosomal membrane, since biochemical studies have shown that there are multiple sequences of peptides that target endosomal compartments. The composition of late endosomes is also fairly complex especially because it involves internal and limiting membranes and this is responsible for obfuscating the exact location of NPC1 [20]. NPC2 is a lysosome protein that is soluble and has a high affinity for binding cholesterol. Unlike NPC1, NPC2 protein is relatively small i.e. 132 amino acids and is transported to the lysosome through the mannose-6-phosphate receptor after which, it binds cholesterol. *In vitro* analysis has shown that this protein can bind to fatty acids with lower affinity [21-23].

When the ligand free crystal structure of NPC2 is analyzed, it can be seen that there three hydrophobic cavities which form a sort of a gate. This gate can expand or dilate to let cholesterol molecules pass through contributing towards cholesterol trafficking alterations [24]. Even though the exact functions of the proteins, NPC1 and NPC2 remain unclear, when double mutant mice i.e. deficient in both NPC1 and NPC2 were compared to single gene deficient mice, it was observed that there exists a cooperativity among the two proteins and they share a common lipid transport mechanism [18, 25-28]. According to the current state of knowledge and in accordance with previous studies, a "handoff" model has been posited in terms of the coordination between the two proteins. According to this model, cholesterol that is released within the lysosome binds to the NPC2 protein with the hydroxyl group exposed. A swift transfer to the N-terminal domain of NPC1 reverses its orientation in a way that the hydrophobic chain may lead the way towards the membrane. Indicate that the role of NPC2/NPC1. However, current evidence hints that retrograde cholesterol transport from the plasma membrane to the endoplasmic reticulum (ER) may not require NPC1 and cellular function still remains unclear [21, 29-32].

1.2. NPC1 and NPC2 within the endo-lysosomal system:

In mammalian cells, Low Density Lipoproteins or LDLs are cholesterol derivatives that act as the primary cholesterol carriers in the blood. These LDLs bind to the LDL receptors and travel to the endocytic compartments of the cell. Cholesteryl esters, the main component of these internalized compounds, undergo hydrolysis to form cholesterol and fatty acids. The enzyme responsible for this hydrolysis is acid lipase. *In vitro* observations show that most of the acid lipase is in separate endocytic compartments that are different from late endosomes or lysosomes and after the enzyme performs its action the free cholesterol then goes to the late endosomes or lysosomes [33]. For cells that are affected by the NPC1 mutation, the cholesterol transport from these late endosomes to several destinations is altered. Even though it is known that NPC1 and NPC2 work together to transport cholesterol, it is not quite known how they perform this function [34].

Cholesterol is synthesized as much in the extrahepatic tissues as it is in the liver. It is observed that in variegated mammalian cells, the biosynthesis of these sterols occur at the endoplasmic reticulum. Following this biosynthesis, most of these sterols are transported from the endoplasmic reticulum to the plasma membrane. This process is energy dependent in nature and also does not depend on NPC1 [35]. Within minutes of reaching the plasma membrane, these sterols recycle between the plasma membrane and endosome. After about eight hours, these sterols accumulate in the endo-lysosomal compartment of NPC1. This accumulation occurs in the NPC1 cells but not normal cells. NPC1 cells exhibit defective processes including the esterification and the recycling of these sterols, from the endosomes, to the plasma membrane [36]. NPC 1 mutation therefore leads to a "trafficking defect" of endogenous compounds such as sterols and engenders several issues. A variety of cells are affected by this NPC1 mutation including macrophages, glial cells and embryonic fibroblasts. Furthermore, the effect on macrophages and glial cells are more pronounced compared to the fibroblasts [37].

1.3. NPC in the brain:

To investigate NPC pathology in the brain, murine and feline*npc1* genes have elucidated plausible mouse models for human NPC [38]. In the mammalian brain, the amount of unesterified cholesterol, which comes from endogenous synthesis, is higher compared to any other organ system [39]. In this regard, neurons and astrocytes have shown to exhibit the trafficking defect of these sterols

as well [40]. These astrocytes however, are still observed to secrete NPC2 proteins, E proteins and apolipoproteins regardless of these specified defects. Cholesterol is not the only lipid that accumulates in NPC1 however; gangliosides (glycosphingolipids present in cell membrane at high concentrations) i.e. GM2 and GM3, sphingomyelins, glucosylceramides and lysobisphophatidic acids also undergo this accumulation process. Genetic mutation related to the catabolism of certain glycoshingolipds often cause glycosphingolipids to accumulate in lysosomes. This in turn leads to secondary accumulation of cholesterol exacerbating the cholesterol trafficking defect [41-44]. Glucosylceramide synthetase is a key enzyme involved in the biosynthetic pathway of gangliosides in eukaryotic cells. N-Butyl deoxynojirimycin (NB-DNJ) may inhibit this enzyme and, in particular, for NPC1 cells, some endosome malfunction may be corrected by treating these cells with NB-DNJ. This line of therapy is further elaborated on this review at a later section, however, it has shown little corrective effect in reversing the cholesterol trafficking defect [45].

A relatively reasonable conclusion that can be made based on these observations is that it is very unlikely that these trafficking defects in NPC1 can be appropriated to glycosphingolipid accumulation. Sphingolipids and cholesterol have high affinity between each other and are major components of lipid micro-domains or “lipid rafts”. The glycosphingolipid accumulation in NPC1 may be explained by these micro-domains since accumulation in one micro-domain in the late endo-lysosomal system may lead to accumulation on another “lipid raft” [46, 47]. Furthermore, it has been demonstrated that in NPC1 cells, endo-lysosomal cholesterol build-up may cause some inhibition of sphingomyelinase and glucosylceramidiase in lysosomes. The latter is responsible for the degradation of sphingomyelin and glucosylceramides. When there is cholesterol loading in NPC1 cells, there is an aberrant localization of glucosylceramidase leading to a lower activity. Other than cholesterol trafficking defect, NPC1 may also be linked to sphingolipid recycling as well. Yeast studies show that mutation in the SSD of NPC1 may result in a defect of this recycling process. This may lead to an alteration in the localization and quantities of glycosphingolipids without changes in sterol metabolism [48, 49]. Important pathological features include prolific growth of ectopic dendrites, formation of meganeurite, neurofibrillary tangles, neroaxonal ataxia and neuroinflammation[16, 26, 42]. In terms of treatment, the most promising trends have been observed in the line of slowing disease progression rather than harnessing a cure. There are a number of experimental treatments such as using neurosteroids and Miglustat that have shown to be in positive light in cell culture and animal models discussed at length at a later section of this review.

2. Experimental study of the neuropathology of NPC:

Before any treatment can be effective in human patients it is necessary to study potential therapeutic agents and targets for NPC in cell culture and animal models. Animal models provide a rather safe and close estimation of results that may possibly be translated to humans. The NPC1 mouse model (BALB/c $NPC1^{NIH}$) has a very well-defined mutation on the *npc1* gene and they are shown to exhibit phenotypes which fairly accurately mimics human NPC disease. A noticeable characteristic of NPC disease is that the Purkinje neurons of the cerebellum are fatally affected; this can be observed in one study where 30-postnatal day NPC1 mice were examined [50]. There are other abnormalities that have been reported in NPC1 mice; for example, in the same study, for 9-PND mice (NPC1 mice at postnatal day 9), there were some mild abnormalities that were noticed in the cerebral white matter, corpus callosum and the never fibers [50].

Several of the regions of the brain are subjected to neuronal cholesterol. 10-PND mice are shown to exhibit axonal injury and 22-PND mice show cholesterol, GM2 and GM3 accumulation in proliferated astrocytes and various other cells in selective regions [51]. This leads to a cell loss in the corpus callosum and the cerebellum contributing towards progressive neurodegeneration. The cell loss specifically affects astrocytes and Purkinje cells, respectively in those areas. After 10 to 12 weeks these degenerations end up causing death to the test subjects. In a recent 2018 study published in *Scientific Reports*, a group of researchers studied the expression/function of excitatory amino acid transporter (EAAT)-expression and its corresponding effect on cerebellar Purkinje cells on $NPC1^{-/-}$ mice and $NPC1^{+/+}$ mice[52]. It was originally suspected and then partially supported by the data from this group that EAATs (i.e. EAAT1, EAAT2, EAAT4), especially EAAT4,do in fact take part in causing Purkinje cell degeneration leading to a cellular loss in NPC1[52-56].

3. Potential therapeutic approaches for NPC

In the early investigative years, patients were administered experimental treatments such as cholesterol lowering diets and combination treatment with cholesterol lowering drugs namely, lovastatin, cholestyramine, nicotinic acid and dimethyl sulfoxide (DMSO). Even though these drugs significantly improved liver cholesterol storage, they had little effect on the betterment of neurological symptoms exhibited by the patients [57,58]. This rather simple approach proved to be ineffective and since then more complex approaches were investigated. In turn, few promising lines of therapy were identified in order to help NPC patients; these are interventions that work to slow the progression of NPC rather than curing it. After studying the molecular pathology of NPC in cell culture

and animal models, researchers have identified possible therapeutic agents such as neurosteroids, curcumin, cholesterol-binding agents and Miglustat for this very purpose [59].

3.1. Substrate reduction therapy

This approach uses specific agents to targets the metabolic precursors that are known to accumulate in lysosomal storage diseases such as NPC. Since sphingolipids are primary components of anomalous lysosomal fat accumulation, in the case of reduction therapy for NPC, these lipid classes have been targeted. One such agent used for this targeting is known as Miglustat (N-butyl deoxynojirimycin or NB-DNJ). Animal studies showed that the NB-DNJ treatment in NPC1 mice and also cats slowed down the onset of clinically observable neurological symptoms. Also, this increased the lifespan of NPC1 mice by about 25% and decreased pathology in the cells of the cerebellum [60]. When it comes to alleviating neurological symptoms in particular, agents such as Miglustat, which cross the blood brain barrier, may be the only effective means for treatment. Miglustat therapy has been showing promise in a series of clinical settings as well. For example in two patients from Taiwan, who started Miglustat therapy at a very early age, stabilization of neurological symptoms were observed between 6 and 12 months after the initiation of therapy [61].

Another study performed on a Brazilian national of 9 years of age was shown to have positive impact by Miglustat on cognitive function, ataxia and so on [62]. In another separate case report out of Japan, researchers focused on the importance of early therapy initiation of Miglustat and its effects on the patients. For this patient of age 4 months the Miglustat was administered and attenuation of the neurological symptoms along with improvement in pulmonary involvement was observed [63]. It was seen in most cases that Miglustat was well tolerated but the benefits reduced or diminished towards advanced stages of the disease [49].

Currently new methods of assessment are being investigated in order to identify the extent of benefit that Miglustat can generate for NPC. In a recent study in the Journal of Clinical Neuroscience, Transcranial magnetic stimulation (TMS) protocols with neuropsychological and clinical testing were done in order to assess the benefits of Miglustat in an NPC patient. In this study, important parameters such as improved cerebellar inhibition, short-latency afferent inhibition and short interval intra-cortical facilitation provided new insights into the pathophysiology of NPC and an assessment of the benefits of Miglustat treatment [64].

3.2. Treatment with curcumin

In another study, the early development of NPC, sphingosine storage and reduced calcium levels in the lysosome in normal human cells that were exposed to NPC induction were identified. The accumulation of sphingomyelin, cholesterol and glycerosphingolipid was in seen as secondary in this model. The elevation of cytosolic calcium pharmacologically offset the NPC phenotype in several cell models. When NPC1 mice were treated with curcumin, the cytosolic calcium levels elevate and the rate of disease progression was slowed by as much as 3 weeks that corresponded to a 35% increase in life expectancy. The investigators in this case concluded that sphingosine accumulation in the lysosome changes the intra-cellular calcium concentrations and causes anomalous endocytic trafficking [65].

3.3. Neurosteroid therapy

Neurosteroids are steroids made by the brain cells which affect neuronal growth and differentiation. They are also in part responsible for modulating neurotransmitter receptors. 48 to 50-PND NPC1 mice have far fewer of these steroids compared to the wild-type mice. When the steroid, allopregnanolone was administered as a single injection to early postnatal NPC1 mice (7-PND), it was shown to delay the onset of neurological symptoms. This also increased Purkinje cell survival, decreased GM2 and GM3 accumulation and doubled the longevity of NPC1 mice [66].

3.4. Novel therapeutic target - Rab proteins

Lysosomes and late endosomes show bi-directional motility (to and fro movement in between the pericentriolar part of cells and the periphery), which is controlled, in part, by various Rab proteins involved in a number of membrane trafficking events. Proteins known as Rab7 (interacts with earlier endosomes and lysosomes) and Rab4 (interacts with the trans-Golgi) are located in the late endosomes. If mammalian cells are treated with cells deficient in the major late endo-lysosomal membrane protein (Lamp1/Lamp2) they exhibit a reduction in motility of late endosomes which in turn leads to the accumulation of cholesterol and NPC like characteristics. A possible explanation of these events may be various endosomal abnormalities that may lead to the inhibition of Rab7 and Rab4. Rab7, when inhibited, produces a reduction of motility in late endosomes. Interestingly enough, the overexpression of Rab9 corrects the fat trafficking defect in NPC1 cells. This pleiotropic effect may be harnessed to provide new and lifesaving therapeutic treatments for NPC [67].

3.5. β-cyclodextrin therapy:

Cells that lack NPC1 and NPC2 fail to transport LDL-derived cholesterol to the endoplasmic reticulum (ER) for esterification purposes by acyl-CoA acyltransferase (ACAT) and therefore exhibit a cholesterol trafficking defect by amassing LDL in lysosomes. 2-hydroxypropyl-β-cyclodextrin can increase the ACAT-mediated esterification of cholesterol and can ameliorate this defect since the buildup of cholesteryl esters in cytosol is anticipated to be

less toxic compared the buildup of free cholesterol in lysosomes[11, 68-70]. On the other hand, methyl-β-cyclodextrin (MβCD) has been shown to reduce lysosomal cholesterol accumulation and correct trafficking defect in NPC fibroblasts but the pharmacological activity reported by different labs have been different [71]. While the systemic administration of cyclodextrins does aid in reducing peripheral organ cholesterol storage and corrects neurodegenerative phenotypes, it comes with its own set of problems. Due to a low permeability through the blood brain barrier toxicity may lead to severe hearing loss in NPC patients [72,73]. In a recent protracted study by Berry Kravis *et al* the long-term effect of intrathecal 2-hydoxypropyl-β-cyclodextrin treatment was studied for 2.5 to 3 years in human NPC patients. Here, out of the three patients studied, all three showed deterioration in eye movements but no other signs of cyclodextrin induced toxicity. According to the measure of what is known as the Neurological severity score, all three patients showed slight improvements in cognitive functions over the course of the treatment [74].

3.6. Other potential therapeutic approaches:

Studies have also shown that anti-apoptotic agents such as imatinib may exhibit improved Purkinje cell survival rates, improve neurological symptoms and protract life expectancy as well [75]. Some researchers have also noticed partial therapeutic benefits of implanted neural stem cells (NSCs) in the treatment of NPC [76]. In a recent study published in 2017 on Oncotarget, researchers were able to generate neural stem cells using re-programming factors SOX2 and HMFA2 from patient-derived NPC fibroblasts (NPC-iNSCs). These cells were stable and differentiated readily into astrocytes and neurons and so on with the help of valproic acid treatment but still showed signs of cholesterol homeostasis defects [77].

Fibroblasts have been a center of attention for NPC for a while; in 2012, Whermann *et al* studied the incorporation of β-cyclodextrins with several cholesterol lowering drugs such as vorinostat (suberanilohydroxamic acid or SAHA), and panobinostat and observed significant enhancement of their activity and alleviation of NPC phenotype in NPC fibroblast [77-79]. Vorinostat is just one example of histone deacetylase (HDAC) inhibitors which have been shown to have cholesterol lowering effects in NPC fibroblasts. Figure 1 shows the structures of vorniostat and a few HDACs that could possibly lead to a successful treatment avenue for NPC in the future [79,80].

Researchers are also exploring several complex but less invasive alternative treatment options for NPC patients. One approach that could be beneficial for neurodegenerative disorders such as NPC is the induction of macroautophagy[81,82]. The benefits of this approach may be very difficult to predict and remains quite uncertain in the case of NPC. *In vitro* studies have shown that an autophagy inducer such as chlorpromazine in conjunction with low dose cyclodextrins may upregulate autophagy clear cholesterol storage [83,84]. A possible group of targets that are subject to investigation are the molecular chaperones of the Hsp70 family. The involvement of Hsp70 in a pathway that can determine whether or not mutated proteins are degraded or re-folded may play a key role for NPC. It's possible role in stabilizing lysosomal membranes through this pathway make them noticeable targets; studies indicate that Hsp70 may play a critical part in the modulation of expression of NPC1 proteins by promoting their degradation. Moreover, the progressive neurodegeneration in NPC has been shown to be linked to lysosomal membrane permeabilization[85, 86].An interesting demonstration by Nakasone *et al* showed recently that, a small molecule i.e. geranylgeranylacetone induces the expression of Hsp70 in cellular models and increases NPC1-I1061T protein expression but reduces cholesterol trafficking defect [87-89].

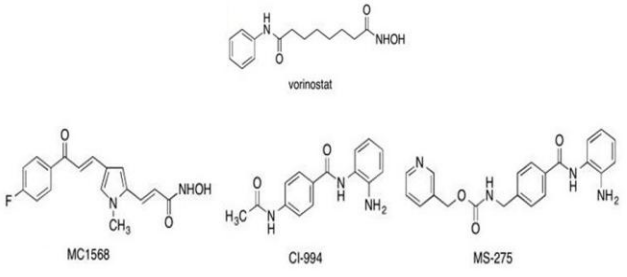

Figure 1: HDAC inhibitors that could possibly be used to treat NPC in the future

Another small molecule known as arimoclomol has also shown similar results and has been already approved for phase 1 clinical trials in NPC patients[90]. Whether these chaperones yield viable results or not still remain to be seen but they may just prove very useful for NPC patients in the near future. Another group of chemical chaperones that have emerged in NPC studies are oxysterols. These are derivatives of cholesterol which are oxygenated and they work by stabilizing proteins through binding to its active site in its native state. Since their small sizes allow for passage through the blood brain barrier, they may be useful in the treatment of neurological disorders such as NPC [91,92].

Studies have shown that these oxysterols have variegated roles in cholesterol metabolism [93,94] and specifically, 25-hydroxycholesterol (25-HC) which has been shown to modulate mutant protein expression levels. Cells that are treated with 25-HC have been observed to correct cholesterol trafficking defect in a dose dependent manner [95]. Of course further studies are warranted with regards to these sterols and special cautions should be taken since the binding of these sterols to proteins are reversible [96]. Furthermore, recent reports have also demonstrated that acetyl-dl-leucine has the ability to improve ataxia

symptoms and quality of life in NPC patients [97] and UDCA (3α, 7β-dihydroxy-5β-cholanic acid) which is a hydrophilic bile acid may be used to improve liver dysfunction in NPC patients [98]. Lastly in a very recent study published on *Experimental Neurology*, Markmann *et al* studied the effectiveness of an adeno-associated virus (AAV), in the treatment of Npc2-/- mice. Compared to untreated mice the AAV-treated Npc2-/- mice showed improvements in disease pathology. Particularly, reduced lysosomal storage, a reduction in Purkinje cell death, reduced gliosis, and improved cognitive performance. In addition, liver and spleen pathology were seen to improve with a marked decrease of liver cholesterol and sphingomyelin. Most importantly, the life span of the mice were significantly extended. All of these taken together the researchers concluded that this AVV, serotype rh.10 gene transfer vector expressing the mouse Npc2 gene (AAVrh.10-mNpc2-HA) was an effective long-term treatment option for NPC disease [99].

4. Roles of other cellular mutations and proteins

Other proteins in the late endosome-lysosome system, such as metastatic lymph node protein 64 (MLN64), MLN64 N-terminal homologue (MENTHO) and Adenosine tri-Phosphate-binding cassette transporter (ABCA1) may also be involved in defects in endosomal cholesterol trafficking but further studies are warranted to identify their extent of involvement [100]. MLN64 and MENTHO has been associated with dysfunction of the mitochondria. Lower expressions of MLN64 have been observed to lower mitochondrial cholesterol in NPC1 cells. The role of MLN64 is not easily understood since studies have shown that mice with mutated MLN64 are often healthy and show minimum alterations in sterol dynamics or in particular cholesterol trafficking [101,102]. In a recent study, Balboa *et al* studied the mechanics of MLN64 in normal and NPC1-lacking cells in order to evaluate the MLN64-dependant mitochondrial functionality changes. The researchers used recombinant-adenovirus-mediated MLN64 gene transfer in order to overexpress the amount of MLN64 in the cells and RNA interference in order to lower the levels of MLN64. The cells overexpressing MLN64 were observed to have a distorted mitochondrial function causing increased levels of mitochondrial cholesterol, higher ATPase activity and reduced glutathione levels, demonstrating the link between mitochondrial cholesterol transport and MLN64 and in turn NPC [103]. ABCA1 is another pivotal protein that, when deficient, cells possess disfigured structures of late endocytic vesicles leading to impaired intra-cellular transport. A mutation in the 3β-hydroxysteroid Δ(7)-reductase gene in fibroblast cells has also shown to accumulate cholesterol in a way that is very similar to NPC1 cells [104]. More recently, NPC cells revealed that ABCA1 has a predilection for recently synthesized sterols before they are internalized by the plasma membrane. Yamauchi *et al* showed that ABCA1 is located inside of a cholesterol-rich membrane domain and newly synthesized sterols such as lanosterol are periodically transferred to this domain. The researchers showed that a significant amount of sterol precursors are moved here and are consequently removed by the ABCA1-dependentpathway. Even though further studies are necessary, this does partially demonstrate the link between ABCA1 and cholesterol trafficking defect [105].

5. Necessity for an interdisciplinary approach to diagnosing adult onset of NPC

Even though there might be an emergence of effective treatments in the near future, the diagnosing of NPC still remains a major challenge. Considering the extremely rare nature of NPC, it can be very difficult to diagnose accurately. This problem is further exacerbated by a wide range of factors that may increase variability; this may include age of onset, clinical emergence, genetic testing and complexities associated with laboratory testing and so on. These have contributed towards the delayed diagnosis of this complex disease and sometimes the knowledge that physicians may have in this area may also play a crucial part. Furthermore, that the clinical manifestation of this disease is also contingent upon patient's age of onset, neurological symptoms, visceral symptoms etc. [106-108] Table 1 shows the neurological and visceral signs that patients may exhibit during each stage of human development in approximation.

Table 1: Neurological and visceral signs during development in Niemann-Pick type C disease [adapted from [107]]

Age range	Developmental Stage	Neurological/Visceral Signs
1 to 3	Early infant	Delay in motor functions/lack of muscle strength
3 to 6	Late infant	Difficulty in walking, delay in speech and cataplexy
6 to 14	Juvenile	Learning difficulty, ataxia, seizures
14 to 27	Adolescent and Adult	Psychiatric problems, ataxia, dystonia and dementia

Researcher Volny et al, presented a case report recently in which the importance of interdisciplinary approaches in case of diagnosing NPC was explored [109]. This particular case report delineates the clinical course and diagnosis in the case of an adult female patient. This patient was identified as compound heterozygote for two different mutations in case of the *npc1* gene. The female patient in question initially exhibited subtle neurological signs at the age of 18. Later on, she showed more pronounced symptoms including deterioration of handwriting and speaking, coughing while eating, memory impairment and static tremors. This patient was first officially examined at

the age of 26 and her physical symptoms were recorded. Afterwards, laboratory tests such as brain Single Photon Emission Computed Tomography (SPECT), magnetic resonance imaging, cerebrospinal fluid analysis, blood cell count, copper and iron levels etc. were performed and most showed normal results. Moreover, genetic testing was negative for Wilson's disease, Huntington's disease etc.

At the age of 26, the first subtle clue was revealed when the patient's abdominal ultrasonography showed a definite hepatosplenomegaly and a biopsy of the liver that revealed mild fibrosis and dilated sinuses. A psychological analysis of the patient revealed a slower psychomotor speed, impaired concentration, decline in working memory and cognitive function. Even though the electrophysiological findings (P300, EMG, EEG etc.) were normal, a depressive syndrome was progressing where the patient's need for antidepressants increased. At the age of 27 of the patient, a trepanbiopsy revealed a permanent splenomegaly.

In collaboration with a histopathologist, NPC was suspected as the primary suspect [110]. After this suspicion, the sequence analysis was done in order to detect mutations in the *npc1* gene and the onset of the disease was subsequently confirmed. This particular case report shows very clearly, the necessity for an interdisciplinary approach when diagnosing NPC. Important fields such as neurology, psychiatry, hematology, histopathology and molecular genetics had to work together in order to identify the culprit in this case that was NPC[111-118]. As mentioned before, the level of knowledge of the physicians, especially neurologists, is also very important since the clinical signs are very subtle at the beginning. As of right now, there is no reliable and easily available method or biomarker by which NPC can be easily diagnosed and further studies need to be done in order to identify them [119-121].

6. Conclusions

Lipid trafficking defects still remain a very complicated problem with several dimensions yet to be explored. Thus, lysosomal storage disorders such as NPC remain elusive to the scientific community. Through the course of this review, it was observed that neuronal and non-neuronal studies carried out on lipid trafficking alterations have revealed that β-cyclodextrin therapy, substrate reduction therapy with Miglustat, and histone deacetylase (HDAC) inhibitors have proven to be the most promising therapeutic agents however, other treatment options are also available. The neurodegeneration resulting from anomalous cholesterol trafficking have severe consequences to the human brain and account for the ultimate death of NPC patients. The grim situation results from mainly twogeneral areas. Firstly, the level of difficulty in diagnosing patients is very high and secondly, no effective long term treatment for NPC identified as of yet [48,59]. Even though agents such as Miglustat have shown promise in terms of slowing the progression of the disease, a cure or more mildly a long-term solution is yet to be shown. The only way to progress this line of research is to identify potential therapeutic targets and use rational drug design to come up with appropriate agents and aggressively combat this disease[122-128]. Therefore, significant work is warranted in every aspect ranging from studying the physiological mechanisms of the disease to designing effective treatment options and hopefully more light can eventually be shed on this rather nebulous problem. Further studies on NPC will not only provide relief to NPC patients but also shed light on other lysosomal storage disorders as well.

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