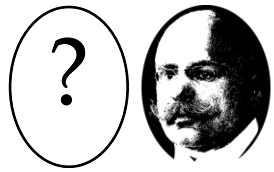
Pfrieger's Digest



Issue 11
March 2024 – July 2024
Niemann-Pick Type A-C

Summaries of latest research advances related to Niemann-Pick diseases, acid sphingomyelinase deficiency (ASMD) and Niemann-Pick type C disease (NPCD), based on selected peer-reviewed publications in scientific journals.

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Dear Readers.
Welcome to the **eleventh** issue covering March 1st 2024 to July 31st 2024. The corresponding links for the PubMed queries are:
- for NPCD:

((niemann-pick c OR niemann-pick type C OR niemann-pick type C1 OR niemann-pick type c2 OR npc1 OR npc2) AND (("2024/03/01"[Date - Publication] : "2024/07/31"[Date - Publication]))) NOT (("2020/01/01"[Date - Publication] : "2024/02/29"[Date - Publication]))

- for ASMD:

((niemann-pick AND ("type a" OR "type B" OR "type A/B") OR smpd1 OR asmase OR acid sphingomyelinase) AND (("2024/03/01"[Date - Publication] : "2024/07/31"[Date - Publication]))) NOT (("2020/01/01"[Date - Publication] : "2024/2/29"[Date - Publication]))

During this period, **71** (NPCD) and **45** (ASMD) articles were published in scientific journals including **7** (NPCD) and **3** (ASMD) reviews. Four articles are related to both areas.

The following statements apply: 1) My selection of articles is entirely subjective. 2) I comment only peer-reviewed articles, and neither preprints nor review articles nor case studies. 3) I only describe articles that I can access or that I receive upon request to authors. 4) I try to ensure correctness of statements, but I cannot guarantee this. 5) My judgements and interpretations are subjective and reflect my personal opinion, they do not claim any validity. 6) I cannot exclude factual errors. 7) I apologize for any errors in grammar, punctuation and orthography, and for any wrong, quirky or otherwise weird expressions. 8) I confirm that the text was generated by myself thanks to my own, evidently limited, natural intelligence without help from any artificial one. 9) This is my translation of my original German version. 10) Feel free to distribute and forward this issue, as long as there are no changes to the text or layout. 11) Translations to other languages are welcome, as long as the original version and my authorship are acknowledged therein. 12) Feedback to: fw-pfrieger@gmx.de.

Patients (NPCD)

PMID:38533577 — Eratne et al. Plasma neurofilament light chain is increased in Niemann-Pick Type C but glial fibrillary acidic protein remains normal

Let's start with a notorious topic: biomarkers. Note that they do not have to be specific for a disease. For specific purposes, its enough if they report the state of a tissue or cell type, in particular the death of nerve cells in the brain, which is evidently difficult to access. Ideally, the markers are present in the blood. The study of the Australian MiND Program ("Markers *i*n Neuropsychiatric Disorders") deals with two proteins, neurofilament, which comes from neurons, and the socalled glial fibrillary acidic protein (GFAP), which comes from a specific type of non-neuronal cells, the astrocytes. Both proteins are part of the cytoskeleton, which – as one may guess – supports the form and stability of cells. It's known since decades that astrocytes produce more GFAP, if things go wrong in the brain. Why is still unclear. The message of the study is clear though: the concentration of neurofilament was elevated in the blood of 11 NPC patients compared to samples from 25 healthy donors. The concentration of GFAP was not. So, neurofilmant seems to be a valid marker for dying neurons.

PMID:39048052 — Mishra et al. Accumulation of alkyl-lysophosphatidylcholines in Niemann-Pick disease type C1

We stay with the topic, and come to a new biomarker that is a bit confusing. It's a lipid named alkyl-lysophosphatidylcholine aka lyso-platelet activating factor. Its concentration is increased in different brain regions of NPC mice and cats compared to healthy animals. But, its concentration in the cerebrospinal fluid of patients was lower than in healthy controls. Why this difference? Unclear. These molecules may react to treatment with cyclodextrin, but the number of patients studied was small. Stay tuned whether the biomarker survives further studies!

PMID:38673803 — Farhat et al. Sterol O-Acyltransferase 1 (SOAT1): A Genetic Modifier of Niemann-Pick Disease, Type C1

A very interesting study, what is it about? Simply about the question, which factors determine when patients show which symptoms and how severe the disease will be. Evidently, the primary factors are specific variants of the NPC1 protein. But this cannot be all. It is known that some siblings with exactly the same variant show different disease onsets and symptoms. Farhat and colleagues confirmed this with 11 siblings. So, what are the other factors that determine the disease onset and progression? It seems really urgent to identify them, as they may also determine the efficacy of therapies. The colleagues from NIH identified a genetic disease modifier, a notorious enzyme named sterol-O-acyltransferase 1 (SOAT1) or acyl-CoA cholesterol acyltransferase-1 (ACAT-1). This protein machine esterifies cholesterol thereby enabling its transport and storage. Previous studies showed that lack of SOAT1 in mice slows down disease progression in NPC1-deficient mice (see Digest #7). That was a first hint. The new study shows that patients with a specific variant of SOAT1 have an earlier disease onset, more neonatal liver disease and more epileptic attacks than their siblings with other SOAT1 variant. Why is this so? Unclear. Evidently, this modifier should be validated using other patient cohorts. But it's a start, and there is more to come.

PMID:38863022	Solomon et al. Swallowing characterization of adult-onset Niemann-Pick, type C1 patients

Another study from the Porter imperium deals with swallowing in 14 patients with adult disease onset, most of them women with an average age of 43 years. The study used standardized tests (ASHA NOMS, NIH-PAS, for experts). The result is clear: all patients in this cohort were able to swallow and breath normally.

Patients (ASMD)

PMID:38592326/	Giacomarra et al. Gaucher Disease or Acid Sphingomyelinase Deficiency? The Importance of Differential Diagnosis

This is about a fundamental question: which disease does the patient have – aka diagnosis. It's known that some diseases present similar symptoms and that some patients get the wrong diagnosis. Italian colleagues have investigated this issue with a large (n = 627) cohort of patients from all over Italy with a suspicion of Gaucher disease. They measured not only the activity of glucocerebrosidase but also of acid sphingomyelinase (ASM), the two enzymes relevant for Gaucher and ASMD, respectively. Indeed, 8 out of the 627 were confirmed to suffer from Gaucher disease. But, heureka, the colleagues also identified 3 ASMD patients, two with chronic neurovisceral form (type A/B), and one chronic visceral case (type B). So the take-home message is "Don't trust your suspicion!"

PMID:38615062 — Mengel et al. A retrospective study of morbidity and mortality of chronic acid sphingomyelinase deficiency in Germany

A Sanofi-sponsored study analysed medical records of 33 German ASMD patients with 24 presenting the chronic visceral and 9 the chronic neurovisceral forms. The charts cover the years 1990 to 2021. The results are similar to those from earlier studies in other countries. Nine patients died in this period, mainly due to liver and lung failure. The five type A/B patients reached a median age of 9 years, whereas the four type B patients died at a median age of 31 years. Naturally, the number of patients is limited, but at least there is now a detailed analysis of the situation in Germany. The data provide a basis to better estimate the efficacy of olipudase treatment in the years to come.

PMID:38739391 — Chang et al. Newborn Screening for 6 Lysosomal Storage Disorders in China

PMID:38992987 — Hickey et al. Newborn screening for acid sphingomyelinase deficiency in Illinois: A single center's experience

Screening of newborns for molecular signs of NPCD or ASMD is an important approach. Two new studies address this topic, the first provides a rare insight in the situation in China, the second looks at Illinois (USA). The results diverge though. Chinese colleagues screened babies born from January to December 2021 in hospitals around Shanghai for six lysosomal storage disorders including ASMD. Out of the 50,108 babies tested, they identified 27 cases with a storage disease and 5 with chronic visceral ASMD. These numbers seem very high for lysosomal disorders in general (1 out of 1,856) and for ASMD in particular with one ASMD Patient out of 10,000 births. The study from Illinois reports a much lower incidence of 0.78 ASMD patients per 100,000 newborns. Stay tuned for studies from other regions and countries!

PMID:38866761 — Sako et al. Allele frequency of pathogenic variants causing acid sphingomyelinase deficiency and Gaucher disease in the general Japanese population

Japanese colleagues studied the frequency of SMPD1 variants (alleles) that can cause ASMD using publicly available genome data. They found that based on the allele frequency, the number of ASMD patients should be one among 128.000, which translates to at least 680 affected persons. According to a poll in 2021, there were only 3 (!) confirmed ASMD patients. There seems to be a diagnosis gap in Japan.

Animal models (NPCD)

PMID:38599016	Goicoechea et al. S-Adenosyl-l-methionine restores brain mitochondrial membrane fluidity and GSH content improving Niemann-Pick type C disease

This is about mitochondria, the notorious "power plants" of cells. They suffer in NPCD, at least in some cells, last not least because their membrane is stuffed with cholesterol. Colleagues from Spain observed in a mouse model of NPC that a specific component, S-adenosyl-L-methionine, is in short supply. This metabolite is required for different reactions, notably the highly popular transfer of methyl groups. Ok, if it's too low, just add more! Indeed, administration of S-adenosyl-l-methionine was good for the animals. It prolonged a bit their life span and diminished a bit the neurologic symptoms. "A bit", it's not a miracle drug. The stuff may be placed on the "mito-booster" shelf, where you also find the long-known N-acetyl-DL-leucine (Tanganil) or its refined version N-acetyl-L-leucine (IB1001).

Cell-based models (NPCD)

PMID:38821960	Kataura et al. Targeting the autophagy-NAD axis protects against cell death in Niemann-Pick type C1 disease models

We know that in NPC specific cells go bust, notably nerve cells. Why is this? There are divergent answers, maybe each cell type dies its own death. Among possible culprits are reduced autophagy and reduced recycling of cell trash causing accumulation of damaged parts, notably mitochondria, and a fading energy metabolism. An international team set out to study these highways-to-hell in different cell models including fibroblasts from mice and humans, and nerve cells derived from stem cells. The group identified two drugs that may help to repair the broken autophagy-mito loop and prevent death of different cell types. One is the FDA approved Celecoxib, which blocks cyclooxygenase 2 and acts anti-inflammatoy. Here, it's unclear how this helps NPC. The other one is called dihydro-nicotinamide riboside, a modified form of vitamin B3. This stuff is required to make nicotinamide-adenine-dinucleotide, which is essential for biological electron transfer. It's a sort of WD-40 that lubes many cellular oxidation/reduction reactions including energy conversion in mitochondria. So, maybe another mito-energy-booster.

PMID:38719150 Brown et al. ORMDL mislocalization by impaired autophagy in Niemann-Pick type C disease leads to increased de novo sphingolipid biosynthesis

This work investigated a little unterstood observation, and delivers a surprising explanation. What's it about? Defects in NPC1 cause accumulation of cholesterol, but also other stuff, including sphingolipids including sphingomyelin. Cells make those in a multistep process using diverse enzymes. The production line is feedback-controled: if too much of the product accumulates, its synthesis is reduced. The first step towards sphingolipids is done by an enzyme called serine palmitoyltransferase. It's made of different subunits and another protein named ORMDL, the latter mediates the feedback control. The colleagues show in different cell models that the feedback fails, if NPC1 is broken. So, sphingolipid material is produced like mad. Why? Because the ORMDL is stuck in broken autophagosomen – why is unclear – and it does not show up at its workplace. Inhibition gone, it's all haywire. Don't we know this from other situations! The work shows further that the above mentioned N-acetyl-L-leucine solves the problem, at least in cells.

PMID:38877068 Lauritsen et al. Ratiometric fluorescence nanoscopy and lifetime imaging of novel Nile Red analogs for analysis of membrane packing in living cells

A contribution from Denmark finally provides an opportunity to cite Goethe "Am farbigen Abglanz haben wir das Leben", no, I won't translate. Or, you can only see (in cells) what you can stain. This is certainly true for fatty substances aka lipids. It is not easy to make them visible. Often, natural substances can be used for this purpose. Filipin from bacteria binds to cholesterol, lysenin from the worm *Eisenia fetida* binds to sphingomyelin. This work aims to study the state of membranes that wrap around cells and their innards, the organelles. The colleagues have chemically modified a long-known stain named nile red. The new molecules allow to examine specific properties of membranes and pathologic changes, for example due to defects in NPC1, albeit with sophisticated light microscopy.

PMID:38886558 Palmulli et al. CD63 sorts cholesterol into endosomes for storage and distribution via exosomes

French colleagues made a very interesting discovery that will surely entail further studies. It's about a well-known protein named CD63. This pops up in many studies on NPC (and ASMD). It serves as marker for the endosomal-lysosomal system of cells. In addition, CD63 is part of extracellular vesicles (s. issue 6 and 9), which continue to be a hot topic. More on that further down. The colleagues studied what happens to cells if they lack CD63. Well, at first sight, relatively little, the publication has a long litany of negative results. As a student, you need guts to get through this! But it was worth to keep going. A very nice experiment showed: if CD63 is missing, the pharmacologic inhibition of NPC1 does NOT cause intracellular accumulation of cholesterol! Wow! The colleagues think that CD63 is somehow involved in the sorting of cholesterol within cells, notably into socalled intralumenal vesicles. These guys populate the endosomal-lysosomal system. If enough of them cuddle together, we call them multivesicular bodies. Remember that NPC2 and NPC1 shove cholesterol out of structures like these. If there's no cholesterol in intralumenal vesicles, it cannot accumulate when NPC1 is broken. The study goes further: the vesicles can be thrown out by cells, then they are called extracellular. The colleagues think that cells use this pathway to carry cholesterol from one cell to the next. This is very exciting, but – all experiments were performed with cell lines. Cell lines are as close to differentiated cells as a stone age stone tool to a fully equipped Swiss army knife. We shall see how CD63 regulates cholesterol distribution in which cells. It would be interesting to know, whether mice lacking CD63 are resistant to pathogenic NPC1 variants. "Allez les bleus!"

Molecules (NPCD)

PMID:38568972 Frain et al. Conformational changes in the Niemann-Pick type C1 protein NCR1 drive sterol translocation

It's time for a new category, molecules. This work is neither about cells nor animals nor patients. It's about the protein, NPC1, or more precisely about the structure of the NPC1 protein in yeast. This one, aka NCR1, is similar to the human version. Now, why care? That's easy: only, if we know how the protein, the machine, looks like, we can understand, how it works; some 500 Million years of engineering, more than the Diesel engine! We have learnt a lot about the NPC1 structure within the last years last not least due to improved methods. The new work by Danish colleagues provides new hints how NPC1 transports sterols. Ultimately, it's all about energy. Energy is needed to heave the cholesterol molecule out of the lysosomal swamp. The new study shows that the energy is drawn from the unequal distribution of positively charged hydrogen, aka protons: there are more of them inside the lysosome compared to outside, the watery cell juice aka cytoplasm. This concentration gradient serves as energy store that is used by the NPC1 protein. Roughly, this goes as follows: A proton binds to a niche within the NPC1 protein. This causes some gymnastic bending of NPC1. This in turn allows the sterol to move on, the proton slips out and the bend snaps back. The cycle can start again. Proton-driven transport machines are a big success in living beings. During evolution numerous proteins emerged that use this mechanism to transport things across membranes. The new study marks a big step forward confirming earlier ideas. As always, not everybody will be convinced, it's a fundamental issue, the plot thickens.

PMID:38686625	Javanshad et al. Endogenous Protein-Protein Interaction Network of the NPC Cholesterol Transporter 1 in the Cerebral Cortex

Another entry in the new category, here the goal was to identify cuddling partners of NPC1. It's known from personal experience that binding partners provide insight into preferences, habits and activities. The same is true for proteins. The Cologna troup delivers another catalog with possible binding partners of NPC1. There are already several lists out there, and in each study new partners are added, and old ones are dumped. The new study is interesting because it fished in nerve cells. And it shows that NPC1 may interact with parts of synapses and of the cytoskeleton. Both are important for nerve cells. We shall see, which partners stand the test of time.

Molecules (ASMD)

PMID:38782304	Scrima et al. ASM variants in the spotlight: A structure-based atlas for unraveling pathogenic mechanisms in lysosomal acid sphingomyelinase

Herculean work from Denmark. The colleagues aimed at all known variants of ASM – there are more than 400 – and tried to find out by computer modeling, how each variant affects the form and ultimately the function of the enzyme. The comprehensive study suggests that many of variants that provoke the severe forms of the disease diminish the stability of the protein provoking its degradation in cells. There are also variants that impair directly enyzme function. Notably, only single variants could be studied, but not the bewildering two-variant combinations in heterozygous patients.

Miscellaneous

PMID:38922499	Kim et al. Transcriptome analysis of East Asian common octopus, Octopus sinensis, paralarvae

Who would have thought? Chinese colleagues report that during the development of the common East Asian octopus (*Octopus sinensis*) embryos, the NPC1 protein is upregulated.

PMID:38856177	Allende et al. Lysosomal cholesterol accumulation in aged astrocytes impairs cholesterol delivery to neurons and can be rescued by cannabinoids

Here's a study from Argentina on specific cells in the brain named astrocytes, a type of non-neuronal cells. They are as important as neurons, but less well studied. The study follows up on previous work (see Guix et al., 2021; Digest #5). It's not really about NPC, but about ageing. Yes, this can be studied, even in cell culture, where cells languish in a warm broth. Reminds a bit of a Perma-Jacuzzi, where all sorts of things may shrink. In this study, ageing was artificially induced – quite the opposite of those artificial rejuvenation attempts that are popular with some humans. Here, the trick was elevated oxidative stress. The study shows an age-dependent decrease of NPC1 protein in astrocytes that causes an accumulation of cholesterol. But, attention, there may be a remedy: cannabis! Treatment of cells with an endocannabinoid or a plant-derived cannabinoid reduced the cholesterol accumulation in ageing astrocytes. Does this phenomenon occur in ageing organisms?