Pfrieger's Digest for Niemann-Pick Type A-C
Summaries of new research advances related to Niemann-Pick diseases based on selected peer-reviewed publications in scientific journals.

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Dear Readers.
Welcome to the eighth issue covering October 1st 2022 to March 31st 2023. 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 (("2022/10/01"[Date - Publication] : "2023/03/31"[Date - Publication])) NOT (("2020/01/01"[Date - Publication] : "2022/9/30"[Date - Publication]))

- for ASMD:

((niemann-pick AND ("type a" OR "type B" OR "type A/B") OR smpd1 OR asmase OR acid sphingomyelinase) AND (("2022/10/01"[Date - Publication] : "2023/03/31"[Date - Publication])) NOT (("2020/01/01"[Date - Publication] : "2022/9/30"[Date - Publication]))

During the period, **69** (NPCD) and **73** (ASMD) articles were published in scientific journals including **9** (NPCD) and **6** (ASMD) reviews. Nine articles belong to both areas.

The following applies as stated in previous issues: 1) My selection of articles is subjective. 2) I do not comment on review articles or case studies. 3) I only describe articles to which I have access or which I receive upon request to authors. 4) I try to ensure correctness of statements, but I cannot guarantee this. 5) My judgements and interpretations expressed in this issue are subjective and reflect my personal opinion, they do not claim any validity and they may be erroneous. 6) I cannot exclude factual errors, 7) I apologize for errors in grammar 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. As for previous issues, this is my translation of my original German version. Feel free to distribute and forward this issue, and to send feedback to: fw-pfrieger@gmx.de or frank.pfrieger@unistra.fr.

https://pubmed.ncbi.nlm.nih.gov/37003582/
First, a "plug". I draw your attention to a review that I have written and finally published in *Progress in Lipid Research* (Pfrieger, 2023). The article aims to provide a sort of synopsis of the Niemann-Pick diseases. This opus had a turbulent, two-years-long history, but it finally ended up in a good journal. The article is freely accessible thanks to the support by the German (Niemann-Pick Selbsthilfegruppe e.V.) and the Swiss (NPSuisse) Niemann-Pick association. I hope it will be useful, at the least it may help to fall asleep.

Patients (NPCD)
https://pubmed.ncbi.nlm.nih.gov/36265573/
A retrospective study from France summarizes data from NPCD Patients, who presented enlarged livers and cholestasis at birth (Gardin et al., 2022 J Pediatr). There have been similar studies, but this one covers a relatively large number of patients (32). Three of the patients died within the first six months after birth due to hepatic failure, for all others, the symptoms dissappeared later on. As shown previously, no clear correlation between severity of liver disease and the occurrence of neurologic symptoms could be established, or between gene variants and disease progression. Based on their experience, the authors advise to measure in patients with neonatal cholestestasis diverse biomarkers (alpha-fetoprotein, oxsterols and N-palmitoyl-O-phosphocholine-serine, PPCS, a.k.a. lysosphingomyelin-509), and to perform immunohistochemical staining of liver biopsies with an antibody against CD68, a marker of activated macrophages. This combination can help to identify patients with a suspicion of NPCD and to subject them to genetic tests.

https://pubmed.ncbi.nlm.nih.gov/36279795/
Hastings and colleagues report results from a clinical trial sponsored by Cyclo Therapeutics (Hastings et al., 2022 Mol Genet Metab). It explored the tolerability of intravenously administrated hydroxypropyl-beta-cyclodextrin. The company calls its specific version of the compound Trappsol. There have been similar studies, but not with adult patients. Thirteen patients received during 12 weeks a total of seven intravenous infusions of Trappsol every 14 days with each session lasting 8-9 hours. Two concentrations were administered, naturally only one per patient. In addition to safety and tolerability, the study assessed the socalled pharmacokinetics and -dynamics: how long does Trappsol stay in the body? Does it do something?

Once again cyclodextrin, the "boomerang therapy" of NPCD. As reminder, cyclodextrins are natural stuff made of sugar molecules that emerge from bacterial

digestion of plant-derived starch – remember those rotting potatoes – but it's not the cyclodextrin that stinks! Because of their special properties, cyclodextrins are everywhere and used by the food, cosmetics, tobacco and pharmaceutical industry. They look like those Italian coffeecups without the strange brown color. They are special, because the are "water-loving" externally, and "fat-loving" internally - similar to the author. Thereby they can carry all sorts of fatty substances through watery milieu, a big challenge in many industrial applications. There are alpha, beta and gamma cyclodextrins that differ in their diameter. Cholesterol fits into the beta (Cappuccino) and gamma (French cafe au lait), but not in the alpha (Expresso) version.

Back to the study: of the 13 participating patients, 10 made it through the trial. The results show that cyclodextrin is rapidly eliminated from the body through the kidney, its blood concentration halves every two hours; that was known already. Some adverse events occurred including the usual suspects like vomiting, headache and nausea, but also hearing decline, which led in two patients to a transient stop of the study. Does it do something? The authors report less cholesterol accumulation in the liver, and a reduction of the previously mentioned biomarker PPCS. What about the brain? Beta-cyclodextrin is too big to cross the blood-brain barrier, and there is no natural transporter. According to the study, a tiny bit trickled into the brain of patients. While the concentration in blood was around 2000 micrograms per milliliter, it reached 40 mg per milliliter in the *liquor cerebrospinalis* – also known as cerebrospinal fluid (see below) – regardless of the dose. So, roughly 2% of the cyclodextrin enter into the brain. The local concentration in region XYZ may be higher or lower. That's not much, and whether this can do something remains unclear. The authors find some changes in brain-derived biomarkers, and they report some improvements in patients. Evidently, all this is still a bit shaky, notably due to the limited number of participants and the lack of a placebo control leaving it unclear whether all of the changes observed were drug-induced.

https://pubmed.ncbi.nlm.nih.gov/36638187/

Next topic, biomarkers: A new study scrutizined the proteins in the cerebrospinal fluid by mass spectrometry (Li et al., 2023 Proteomics). The keyword here is *proteomics*: this means the identification of – ideally all – proteins that are contained in a biological sample (tissue, blood, cells etc.). Mass spectrometry is the method of choice, as it allows to identify proteins and many other molecules; its applications range from detection of doping drugs to explosives. Nowadays, *proteomics* studies of cerebrospinal fluid are *en vogue*, notably in connection with frequent neurodegenerative diseases Alzheimer and Parkinson. Cerebrospinal fluid – why

this fluid? Simply because its composition is thought to reflect ongoing processes in the brain. Humans produce half a liter per day. In principle, this is filtered blood that seeps into the brain in a specific brain area called the *plexus choroideus*. For etymology fans: its a tissue (*plexus*) that looks like (*-ideus*) the afterbirth (*chorion*). The blood-liquor barrier is different from the much tighter blood-brain barrier. About 20% of the cerebrospinal fluid are renewed - per hour, notably during the night, probably because the brain is rinsed intensely while we are asleep. Though this doesn't show every morning – at least in the author's experience.

The new study compared samples from healthy donors and NPCD patients, and tested whether treatment with Miglustat/Zavesca reverts disease-induced changes. The approach identified 300 different proteins in the cerebrospinal fluid, 71 of which changed in samples from patients. Note that 300 are only a fraction of the total number present in this brain soup. Other studies unrelated to NPCD detected five to ten times more proteins. Evidently, the technology like all has sensitivity limits and tends to detect the most abundant proteins.

The study puts several new biomarkers on stage including the neuropeptide Y. That's its name, and no, there is no neuropeptide X, as far as I know. This is a small secreted protein that elicits specific reactions in target cells. One idea is that it protects neurons from death. The amount of neuropeptide Y was enhanced in patients and reduced by Miglustat treatment. Why, is unclear. It may be part of a brain-intrinsic rescue operation, among others it reduces inflammation. One should note that the composition of the cerebrospinal fluid varies from one individual to the other, and it depends on where and when during the day it is tapped. On the other hand, the methods to detect proteins become ever more sophisticated. Therefore, it is likely that more biomarkers will be identified. To be continued in the next paragraphs.

https://pubmed.ncbi.nlm.nih.gov/36721240/

Here we go, another biomarker study, again *proteomics*, and again cerebrospinal fluid (Campbell et al., 2023). This study based on 28 patients and 30 healthy controls is worth mentioning, because it used a completely different technology than the aforementioned work. Its name is *proximal extension assay,* which is sold by a company named Olink. Originally, the technology was developed in Sweden, patents go back to the 1990s. In contrast to mass spectrometry, which shoots at all proteins, this method focuses on defined sets of candidate proteins that are pre-selected by the company. In the NPCD study, four panels of organ- and disease-related proteins were analysed, 1467 in total. The method, which is most suitable for body fluids (urine, blood etc.), is highly sensitive, as it combines several selection and binding principles. The study confirms previously identified biomarkers, such

as calbindin and neurofilament, which are components of nerve cells, and it introduces new ones. The study also tested, whether the amounts of the markers in the cerebrospinal fluid correlate with the disease state of a patient. At least one of these markers did so. A protein with an ugly name *C–C motif chemokine ligand 18* (CCL18) was negatively correlated with the age of neurologic disease onset, meaning: the higher, the younger. Follow-up studies will have to show whether these biomarkers function also in other patient cohorts. One can expect that the intense efforts currently under way will reveal an entire forest of biomarkers. Patients' disease states may then be represented by multidimensional landscapes rather than whirly trajectories of single parameters.

https://pubmed.ncbi.nlm.nih.gov/36470574/
Biomarkers forever! The Porter group looked after a previously mentioned marker, the neurofilament protein. It is part of the internal skeleton of neurons, and it gets spilled into the cerebrospinal fluid, when neurons and their processes, the axons, go bust (see Digest 6). The new study (Agrawal et al., 2023 Genet Med) confirms – based on a large number of patients – that the amount of neurofilament reflects neurologic disease progression. Its amount in the brain soup depends on the severity of neurologic symptoms, and it is modified by miglustat. As in all studies, there are some limits, notably comparisons to "normal" donors: there are no cerebrospinal fluid samples from healthy kids. Evidently, the dream would be to avoid the brain soup tap and to detect neurofilament in blood, which would simplify the whole affair. Stay tuned!

Patients (ASMD)
https://pubmed.ncbi.nlm.nih.gov/36205749/
https://pubmed.ncbi.nlm.nih.gov/36517856/
Diaz and colleagues present in two articles (Diaz et al., 2022 Genet Med; Dias et al., 2022 Orphanet J Rare Dis) the results of a multinational olipudase alfa study in 20 patients under age 18 presenting chronic ASMD, which was sponsored by Sanofi (ASCEND-Peds study). The enzyme ASM was administered every two weeks by intravenous injections. The study should show whether the treatment is safe, whether it provokes adverse events, and whether it helps. To make it short: it works, and all patients finished the study. The most frequent adverse events were fever, urticaria and rash, headache and vomiting. In some patients, the treatment had to be briefly interrupted or a specific dose had to be repeated. The adverse events diminished during the course of the treatment.

As reminder: olipudase alfa increases the concentration of ceramide, which is a product of enzymatic sphingomyelin breakdown. The molecule in turn can enhance

inflammation. Therefore, the dose of olipudase is stepwise enhanced during the treatment.

Examination of most affected organs (spleen, liver, lung) showed positive effects. Olipudase alfa diminished the size of spleen and liver within the first six months, later on the organ sizes remained stable or diminished further. Lung function could be measured in nine patients: they all showed improvements. Treated patients also exhibited enhanced body growth. Last not least thanks to these results, the drug is now approved in many countries worldwide.

https://pubmed.ncbi.nlm.nih.gov/36348386/
A study from Brasil deals with two "old" biomarkers (Kubaski et al., 2022 Orphanet J Rare Dis). The authors' analyses show that lysosphingomyelin, a sort of amputated sphingomyelin, together with PPCS can be used to diagnose ASMD patients. Apparently, this also works using dried blood spots.

Animal models (NPCD)
https://pubmed.ncbi.nlm.nih.gov/36322609/
A study from Italy describes pathologic changes in the cerebellum and olfactory bulb (smelling!) of mice with a defect in NPC1 (Rava et al., 2022 J Cell Physiol). Here, we are not talking about the knockout mouse, which lacks NPC1 completely and dies at 11-12 weeks of age. The mouse used by Rava and colleagues, bearing the allele code *nmf164*, has a chemically induced variant of NPC1, where amino acid number 1005 has been modified (D1005G). These animals show slower disease progression than the ko mouse. The group focused on microglial cells, which were mentioned in previous issues. As reminder, these cells function as sort of ambulance, firepolice, and garbage collectors. The results reveal that the cells are already activated and start to clean up stuff, when mice are only two weeks old, which is well before the onset of symptoms. Moreover, the mice show defects in olfaction at the age of six weeks, which is still before neurologic symptoms like ataxia occur. It's unclear though whether activation of microglia is good or bad. This needs more work. Is the sense of smelling affected in NPC patients?

https://pubmed.ncbi.nlm.nih.gov/36455410/
We stay with mouse models for NPCD, but switch to the *knockout*. A new study reports effects of bimoclomol, a sibling of the well-known arimoclomol (Gray et al., 2022 eBiomedicine).

Talking about timing: the publication of research results often resembles a seemingly endless roller-coaster trip, in other words, it can take forever, and meanwhile reality (and competitors) pass by. The "history" of scientific publications

is usually indicated somewhere at the beginning or end of an article. It is written when the article was first submitted (in this specific case February 2020), when the revised version with the changes demanded by referees was submitted (October 2022; two years later!), when the article was finally accepted by referees (November 2022), and when it was finally published (November 2022, that was fast).

The present study follows up on previous work published in 2016 showing that intraperitoneal injection of recombinant – meaning industrially produced – *heatshock protein 70,* improves the isolation (myelin sheath) surrounding neuronal cables (axons). The new work shows that this works also with bimoclomol. Injection of the drug enhanced the amount of specific components of the cable isolation. Remember that bi- and ari-moclomol are thought to act as *heatshock protein* activators thereby affecting probably a large number of cellular components. How this works is unclear, and it is unclear, whether these effects occur in patients.

https://pubmed.ncbi.nlm.nih.gov/36301667/

The next article dampens a bit the enthusiasm for a specific mouse model (Schultz et al., 2022). The topic of interest is the notorious I1061T variant of NPC1, which is present in many patients. Previous studies showed that it functions, but doesn't pass the cellular quality control system, it ties up and gets degraded before it reaches its workplace, the endosomal-lysosomal system. The finding provoked the search for a sort of therapeutic bodyguard & escort service (the correct term is *proteostasis*, but that sounds awful) helping the protein to escape degradation and to reach the lysosome. The keyword here: chaperones. To test these approaches, a mouse was created, where the I1061T variant was introduced into the mouse version of NPC1 – sounds easy, but it's a lot of work. Now, many drug tests did not show effects in this model. Why? The new study suggests: "It's the mouse, stupid!". The somewhat artificial mouse variant behaves differently compared to the human I1061T variant. It is also degraded, but a large fraction gets to the lysosome, where it putters around. Therefore, cholesterol accumulation and overall disease progression are relatively slow. A reason for this distinct behaviour could be specific parts of the NPC1 protein, where the sugar icing differs between mouse and human. So, in a way, the mouse is still useful, but not necessarily to test modifiers of proteostasis.

https://pubmed.ncbi.nlm.nih.gov/36614015/

A data-heavy study compared the wet weights of organs in normal and NPC1-deficient (ko) mice separating male and female animals. Moreover, the authors tested the effects of several treatments on these values (Antipova et al., 2022 Int J Mol Sci). On offer: miglustat, cyclodextrin and a combination of miglustat, allopregnanolone and cyclodextrin. A total of 92 (!) measures were collected. This

industrious work revealed sex-dependent and -independent differences in the weight of organs and different effects of miglustat and cyclodextrin or combinations thereof – allopregnanolone did not show effects. Just an example: the liver was heavier in NPC1-deficient mice compared to healthy littermates regardless of sex, whereas the spleen weight was increased only in males. The differences were eliminated by cyclodextrin, but not by miglustat. The authors generously summarized their pile of data in tables.

https://pubmed.ncbi.nlm.nih.gov/36861884/
A new player may have entered the NPCD field, a big protein named STARD9 (Sterling et al., 2023 J Cell Biol). This is a bit curious, because according to the literature, the protein harboring a motor plays a role in cell division. The new work suggests that the protein also mediates some sort of intracellular gymnastics. In one exercise, observed in cells growing on a culture dish, lysosomes grow tube-like structures that seem to be full of NPC1 protein. Sense or nonsense – unclear for the moment. STARD9 seems to participate in this gym stretch exercise. If STARD9 is missing from cells, cholesterol accumulates, if it's missing from the mouse, Purkinje cells go bust and neurologic symptoms ensue. It remains to be seen whether and what this has to do with NPCD.

Animal models (ASMD)
https://pubmed.ncbi.nlm.nih.gov/36951087/
This study asks what happens if you remove not only SMPD1, but also GBA1 (Keatinge et al., 2023 Dis Model Mech). Sounds cryptic? Carrying two variants of SMPD1, one per chromosome (homozygosity), provokes ASMD. However, carrying one variant alone (only one chromosome, heterozygosity) can increase the risk of Parkinson's disease. Curiously, this is similar to a different lysosomal enzyme called *glucocerebrosidase beta 1* (GBA1). Two broken variants and the carrier presents Gaucher disease, one alone increases the risk for Parkinson. Nobody knows why, but there are lots of ideas. What can be done? Ask the fish, this is a classic case for fish. Not any fish, mackarel, trout or those frost-resistant stick fish in your freezer, but the favorite fish of biomedical researchers (and aquarium lovers): *Danio rerio* a.k.a. as zebrafish. Small, spry, frugal, and with all the bells and whistles of a vertebrate including a nervous system, and most importantly, easily modifyable – genetically. The authors generated fish that lack both GBA1 and SMPD1 to test, whether lack of one enzyme worsens the lack of the other one. But, as often in biology (or life), it didn't turn out as expected. Fish lacking SMPD1 and GBA1 were much better than fish lacking only GBA1. The motor defects were gone, and they lived longer.

Somehow, the lack of SMPD1 seems to repair the defects due to GBA1 deficiency, notably in mitochondria and lysosomes. How, is unclear.

Cell-based models (NPCD)

https://pubmed.ncbi.nlm.nih.gov/36447062/
https://pubmed.ncbi.nlm.nih.gov/36795166/

Colleagues from Brasil studied fibroblasts from skin biopsies of patiens, and tested whether antioxidants like coenzyme Q10 und N-acetylcysteine (not to be confounded with N-acetyl-DL-leucine) reduce the intracellular accumulation of cholesterol (Hammerschmidt et al., 2023 Metab Brain Dis; Hammerschmidt et al., 2023 Naunyn-Schmiedeberg's Arch Pharmacol). Indeed, they did, but not much. There have been previous studies on this topic, but overall this antioxidant business remains cumbersome: there is something going on in cells, but what in which cells, and how to treat it. This needs more work ideally in animal models.

https://pubmed.ncbi.nlm.nih.gov/36893262/

New insight and progress require new tools. This becomes evident whenever you try to hang something on a concrete wall with a hammer and a nail. There are similar cell biological problems: how can one know, which lipid is when and where in a cell and gets involved with which protein. Most fatty substances do not blink, and they are relatively difficult to handle, a good example is cholesterol. Based on previous work, the Höglinger troup has developed a sort of multifunctional Leatherman tool for lipids (Altuzar et al., 2023 PNAS). To this end, selected lipids are equiped with hooks, bulbs, pliers, even a sort of magic cap. Once this dolled up lipid is inside cells one can switch on the bulb, and track the stuff. Or one can use the hook to pull down binding partners, and one can remove the magic cap and test how the cell deals with the lipid. The authors find – quite surprisingly – that NPC1 may not only transport cholesterol but also a molecule named sphingosine. The latter is an elementary component of sphingolipids including sphingomyelin. Although ways how cells synthesize and degrade sphingolipids are well known, it is unclear, how sphingosine gets out of the endosomal-lysosomal system. Is there a specific transporter? The new study suggests it is NPC1. That's very interesting, and future work will show whether it's true.

This kind of studies provokes a notorious comment. Chemists like to produce stuff like the Leatherman tool that are eagerly used by biologists. However, a question is to which degree these artificial molecules behave "naturally" without disturbing the cellular peace. An often used argument is that these molecules are only "slightly" modified, and therefore should not disturb too much. This seems a bit simplistic, if one accepts that each natural lipid is the product of a billion year or so

of engineering optimized to fullfill its function. There is no carbon, hydrogen or whatever atom or double bond too much or too little. Therefore, it seems daring to assume that these artificial constructs behave "naturally". Nevertheless, this does not exclude that the new tools enable discoveries and bring about new insight – as long as the results are subsequently validated by independent methods in "real" cells. In the specific case, one would like to know whether sphingosine accumulates in highly specialized cells, like neurons, from NPC1-deficient animals. Progress on the painting-concrete-wall front requires a hammer drill, anchor bolts, and screws – or a different wall.

https://pubmed.ncbi.nlm.nih.gov/36823305/
There's news about a peculiar component of cells with two similarly bulky names: bis(monoacylglycero)phosphate (BMP) or lyso-bisphosphatidic acid (LBPA; s. Digest issue 5). This funny molecule lives in late endosomes. With its special form, it probably helps to charge NPC2 with cholesterol, and it may help to suck sphingomyelin out of membranes allowing its degradation by ASM. In any case, it has remained a mystery how cells produce this BMP/LBPA stuff. The new work hints to an enzyme, the socalled *lysosomal phospholipase A2* (Chen et al., 2023 Commun Biol). So what? This is of interest for NPCD (and possibly ASMD), because previous work showed that the increase of cellular BMP/LBPA levels can reduce the lipid accumulation due to NPC1 deficiency. So, the new enzyme may become a new therapeutic target for NPCD.

Miscellaneous (NPCD)
https://pubmed.ncbi.nlm.nih.gov/36920643/
News from the insect imperium. The commercially available (600 pieces for 16.95$), but barely storable (!) predatory mite *Phytoseiulus persimilis* loves to gobble up spider mites. If you deprive it from its version of NPC2 protein, it cannot smell odors that plants produce after being infested by spider mites (Zhou et al., 2023 Exp Appl Acarol), and biological pest control breaks down.