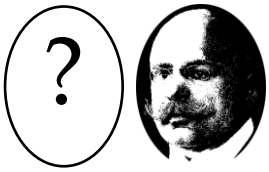
Pfrieger's Digest



Issue 9
April 2023 – September 2023
Niemann-Pick Type A-C

Summaries of latest research advances related to Niemann-Pick diseases (Acid Sphingomyelinase Deficiency, Niemann-Pick Type C) based on selected peer-reviewed publications in scientific journals.

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Dear Readers.
Welcome to the ninth issue covering April 1st 2023 to September 30st 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 (("2023/04/01"[Date - Publication] : "2023/09/30"[Date - Publication]))) NOT (("2020/01/01"[Date - Publication] : "2023/3/31"[Date - Publication]))

- ASMD
((niemann-pick AND ("type a" OR "type B" OR "type A/B") OR smpd1 OR asmase OR acid sphingomyelinase) AND (("2023/04/01"[Date - Publication] : "2023/09/30"[Date - Publication]))) NOT (("2020/01/01"[Date - Publication] : "2023/3/31"[Date - Publication]))

During this period, **78** (NPCD) and **52** (ASMD) articles were published in scientific journals including **10** (NPCD) and **5** (ASMD) reviews. Three articles were related to both areas.

As for all issues, the following applies : 1) My selection of articles is subjective. 2) I do not comment on review articles or 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 and they can be erroneous. 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. As for previous issues, this is my translation of my original German version. Feel free to

distribute and forward this issue. Feedback to: fw-pfrieger@gmx.de or frank.pfrieger@unistra.fr.

Patients (NPC and ASMD)

https://pubmed.ncbi.nlm.nih.gov/37155167/

Let's start with a topic that relates to NPCD and ASMD, the newborn screen. As you know, there are ambitions to include both conditions in these screens. But the issue is much broader, as the efforts apply to many diseases. The authors have polled US experts for rare diseases (Gold et al. 2023 JAMA New Open). Specifically, it was about gene sequencing and the question which genes should be included in the screening panels. Out of 386 experts polled 62 answered, that's a lot, possibly because there was a 50$ gift card, or because the experts were repeatedly solicited. Here some results: a large majority (88%) voted to include monogenetic diseases with a treatment in the screens. Accordingly, *SMPD1*, the gene encoding ASM, is in the top 25 list probably because the treatment with olipudase alfa (Xenpozyme, recombinant ASM) was approved in 2022, when the poll was run. In the case of NPCD, all the same 69% of the experts voted for inclusion in the screen. Besides, only 28% of experts voted to test for diseases lacking a treatment or guidelines for clinical management. Suprisingly, only a very small number of neurologic diseases should be screened for according to the experts. Evidently, the poll has some weaknesses, for example the restriction to the US and a potentially biased selection of experts. But it's good that the subject was polled.

Patients (NPC)

https://pubmed.ncbi.nlm.nih.gov/37210540/

A study from Harvard Medical School in Boston together with the National Niemann-Pick Foundation summarizes the experience of 19 NPCD patients aged 15 to 63 years and their caretakers (Golden et al., 2023 Orphanet J Rare Dis). Evidently, this won't be new for those directly and indirectly affected by the disease. But such studies are very important for the industry (planning of clinical studies), for agencies like the FDA, and for insurances. The interviews showed, not surprisingly, that the progressive cognitive impairment, the loss of autonomy and mobility and the problems with swallowing are particularly cumbersome and demanding. Moreover, the endless search for a proper diagnosis (taking on average 7 years) and the limited treatment options. The poll revealed the willingness of patients to participate in clinical studies, but also the considerable logistic difficulties (transport, cost) that are associated with. This demands careful planning and adequate support. The poll further revealed inequal access of

patients to clinical information and medical care causing concerns and problems to handle the complex disease.

https://pubmed.ncbi.nlm.nih.gov/37353388/
A study by Turkish colleagues exemplifies how to obtain a diagnosis by a detour and state-of-art methods (Capan et al., 2023 Seizure). Their starting point were patients with epileptic encephalopathy. They suffer from brain damage provoked by frequent and treatment-resistant seizures. For most patients, the underlying causes are unknown, some rare diseases may hide in the bushes. Within the framework of a larger project whole-exome sequence data of 29 patients were acquired. As reminder, this method assesses a small but precious part of the genome, the one that codes for proteins. The large rest of the genome is also important, but by miles more complex and largely *terra incognita*. By sequence analyses and simulations of protein structures, the colleagues detected new variants of NPC2 in two sisters. Further genetic testing of the family showed that a third sister with a mental handicap is also homozygous for one of the mutations. So, the yield of these efforts is small in terms of patient numbers, but very important.

https://pubmed.ncbi.nlm.nih.gov/37201244/
The team of Elisabeth Berry-Kravis summarized the long-term experience with lumbar puncture of NPCD patients (Albert et al., 2023 Pediatr Neurol). Out of 59 patients (age 1 to 31), who were treated for up to nine years with between one to 210 (!) infusions, 56% showed no adverse events. On the other hand, only 3% out of nearly 3000 lumbar punctures performed in the context of the Adrabetadex (beta-cyclodextrin) study provoked adverse events. This includes headache (22% of patients), vomiting (24%) and back pain (15%). There was no correlation between the occurrence of adverse events and either the number of punctures or the age of patients, but certainly with the patients' body-mass index. Interestingly, a few patients seemed particularly sensitive to lumbar punctures.

https://pubmed.ncbi.nlm.nih.gov/37348967/
Let's move on with iron, iron in the brain – yes, it's there, like zinc and copper (no gold though, so mining or recycling won't pay off). Now and then, there have been cues that the iron metabolism is bent in NPCD. This was looked at by the Walterfang troupe in Australia. They examined 10 adult NPCD patients and age-matched controls using advanced magnet resonance tomography (Ravanfar et al., 2023 AJNR Am J

Neuroradiol). The latest super-duper machines (Siemens!) and analyses allow to measure the iron distribution in the body, at least indirectly and relatively. The results show that a particular region of the brain, known as the thalamus, is smaller in NPCD patients – this was known – and that iron accumulates in a small part of this area. The enrichment seemed to correlate with the severity of neurologic symptoms (the more the worse), although this did not apply to all severity scales tested. Evidently, the number of patients is small – necessarily. Still, some nice pioneering work going on downunder, there is hopefully more to come. Btw, deleterious effect of iron on cells are undisputed, there's even a specific term for this "ferroptosis". Whether this plays a role in the context of NPCD needs to be studied further.

https://pubmed.ncbi.nlm.nih.gov/37480097/
Back to an "old hat": Miglustat/Zavesca? Déjà-vu? Ever heard of? A French team performed a socalled retrospective study, put it simply, they analysed medical charts from 1990-2013 (Freihuber et al., 2023 Orphanet J Rare Dis). The topic was effects of Miglustat in patients with the early infantile form, where neurologic symptoms occur before the second year of life. You may assume there are lots of studies on this topic. Think again! There are very few. The authors found 26 patients, 10 of them were treated with Miglustat whereas the others were not treated serving as control group. Here's the point: in this patient group, Miglustat doesn't help much, neither for neurologic symptoms nor for the life span. On average, treated kids lived a year longer than those without treatment. However, in the latter group, four children could not be followed up and their fate remained unknown.

https://pubmed.ncbi.nlm.nih.gov/37517328/
Let's continue with a perennial topic, biomarkers. The Porter troupe identified a new biomarker in cerebrospinal fluid. It goes by the bulky name *ubiquitin C-terminal hydrolase-L1* or UCHL1 (Cawley et al., 2023 Mol Genet Metab). The protein helps with the degradation of broken proteins in cells, and pops up since the 2000s in the context of diverse diseases including Parkinson, Alzheimer and stroke. In the brain, it seems to be used by nerve cells only. The results show that the amount of UCHL1 in the CSF of patients is much higher than in healthy donors, that it increases with the disease severity, and that it is diminished by Miglustat. Evidently, it would be great, if the stuff would show up in blood to simplify measurements. Interesting work in progress.

https://pubmed.ncbi.nlm.nih.gov/37670901/
More news concerning the clinical phase I/II trial with intravenous infusion of beta-cyclodextrin called Trappsol (Sharma et al., 2023 Mol Genet Metab Rep). The trial was sponsored by Cyclo Therapeutics (see last issue) and comprised patients from different countries (Israel, UK, USA and Sweden). The patients received over the course of 49 weeks every two weeks eight-hours long intravenous injections of cyclodextrin at three different doses. During this time, all sorts of things were measured to assess pharmacokinetics (Where is cyclo when and for how long; blood, cerebrosinal fluid), pharmacodynamics (does it do something?) and effects on neurologic symptoms. Nine of 12 patients aged 2 to 39 years stayed until the end, distributed over the three doses, so not many left for each dose, five for the lowest, four for the middle and three for the highest. There were no serious adverse events, cyclo remains in the blood for a few jours before it is excreted. Some of it reaches the brain as indicated by its presence in the cerebrospinal fluid. There were positive effects on biomarkers, but the changes in neurologic symptoms are quite mixed, improvements in some domains, impairments in others, depending on the patient. It looks a bit roulette-like, last not least because it is unknown whether patients without treatment or with placebo (not used in this study) would have evolved similarly. We shall see where this approach will be going.

https://pubmed.ncbi.nlm.nih.gov/37433892/
A fairly comprehensive study with 602 (!) NPCD patients is reported by Centogene in Rostock (Germany). The company diagnoses rare diseases genetically and "biomarkerly", and therefore it sits on a large database (Moreno et al., 2023 Eur J Human Genet). For this study, they queried their treasure trove for NPCD digging out data from 2006 to 2021. The patients ranging from newborn to 60+ come from 47 countries all over the world, most from Middle East, Latin America and Africa, regions that are often underrepresented in studies. What's new? The study uncovers 73 new variants of the NPC1 proteins, some of which are "private", i.e. they occur only in single patients. Moreover, the frequency of specific variants varies regionally and blood concentration of PPCS, the marker with the awkward name (see previous issues), correlates with the age of diagnosis reaching a maximum in patients with "loss of function" variants. Moreover, the PPCS concentrations were higher in patients from Middle East, Asia and Africa compared to those from Europe and Latin America. Finally, the data allowed for some cross-correlations between specific variants and symptoms (socalled genotype-phenotype relations). However, the company had limited information about symptoms and none about the age of symptom onset. Still, the amount of data is impressive. In

case any good fairy (or similarly qualified "being") is listening: one wish: that these data together with other information are integrated in an unified and accessible database. This would greatly boost research (and development) in the field.
https://pubmed.ncbi.nlm.nih.gov/37740198/
French colleagues looked into medical charts of NPCD patients with the adolescent/adult form (Morin et al., Orphanet J Rare Dis). With the aim of enabling a timely and reliable diagnosis of this form they asked whether and when which behavioral changes occur in the patients in addition to or instead of psychoses. The authors analysed medical charts of 19 patients from the Center of Lysosomal Diseases at the Pitié-Salpêtrière hospital in Paris. Ten patients showed psychoses including delirium, hallucinations and delusions. Regardless of these, all patients showed a long list of behavioral changes in bewildering combinations including depression, anxiety, emotional hyper reactivity, excessive spending, impaired social cognition, apathy. Some of these changes are indicative of frontal lobe dysfunction. Most patients did not respond to "psychotropic" drugs. Overall, it seems advisable to exam patients showing combinations of the listed behavioral changes for NPCD.

Patients (ASMD)
https://pubmed.ncbi.nlm.nih.gov/37098529
Lachmann and colleagues (Lachman et al., 2023 Orphanet J Rare Dis) report in a Sanofi-sponsored study how olipudase alfa affects patients with chronic visceral form of ASMD (Niemann-Pick Typ B) after long-term treatment for six and a half years. The patients, three men and two women, were between 22 to 47 years old, four received the same dose throughout, whereas one patient received transiently a lower dose. All patients showed adverse events that were classified as mild, one patient showed serious events probably unrelated to the treatment. Overall, the outcomes look very good. However, as one can imagine, the situation of patients with respect to symptom severity was highly diverse before start of the treatment: here a pathologic enlargement of the spleen, there the liver, then a nearly normal lung function and normal bone density or serious lung disease and progressive osteoporosis. The treatment decreased symptoms in all cases, some patients achieved almost normal values with respect to organ volume or function. Interestingly, the values continued to improve throughout the treatment. For example, the liver volume decreased even after several years, less strongly than during the first months, but still steadily. Even bone density improved, albeit slowly. Evidently, the number of patients is very small, but the results demonstrate safety and efficacy of the treatment, which has been approved in 2022.

https://pubmed.ncbi.nlm.nih.gov/37246980/
Just to mention this here: the enzyme ASM emerges in divergent biomedical contexts ranging from depression, arthritis to tumor formation. Take as an example a study from the highly active Gulbins troupe (Wilson et al., 2023 J Mol Med). Their mouse and patient data suggest that ASM activity correlates positively with survival rates following pancreatic tumor. Treatment with olipudase alfa may show benefits in patients suffering from this lethal disease.

https://pubmed.ncbi.nlm.nih.gov/37453187/
Now, biomarkers for ASMD with an old acquaintance from the NPCD world: the protein with a terrible name *glycoprotein non-metastatic protein B* or GPNMB. Apparently, it was also baptized once osteoactivin, this is shorter, but doesn't sound much better. The protein is produced and released by macrophages and microglia, cells from the immune system. It seems to inhibit inflammation and help to repair broken lysosomes. In earlier studies it emerged as biomarker for NPCD (s. issue 6), but also for Gaucher disease. The new work (Eskes et al., 2023 Mol Genet Metab) shows for the first time that the blood concentration of GPNMB Konzentration is elevated in patients with chronic-visceral ASMD (a.k.a. Niemann-Pick typ B) and correlates with disease severity. There'll be more studies with more patients to consolidate these findings.

Animal models (NPCD)
https://pubmed.ncbi.nlm.nih.gov/37024714
Kim and colleagues colleagues (2023 Sci Rep) investigated in a NPCD mouse model carrying a chemically induced variant of NPC1 (NPC1^{nmf164}), whether anything goes wrong in the brain during the first weeks after birth. Here, the brain is still under development. The study focused on the famous Purkinje cells in the cerebellum, which are notoriously vulnerable to defects in NPC1 function. Indeed, the authors revealed erroneous development of cellular structures, notably in dendrites and synapses. Dendrites showed abnormal growth, less mitochondria and lysosomes, and a imbalance of metabolic pathways that are intimately linked to the lysosome (for pros: TFEB and mTORC). It remains to be seen whether and how these early pre-symptomatic changes contribute to the progressive loss of these neurons starting few weeks later.

https://pubmed.ncbi.nlm.nih.gov/37128603/
A study by Chinese colleagues is all about lithium (Han et al., 2023 iScience). Lithium? Lithium? What was it about: electric car batteries, bipolar disorder, and? Short historic

detour: the first PubMed articles about lithium date back to the 1870s, so 150 years ago. Back then, it was reported that the metal shows therapeutic effects for epilepsy, neuroses, sleeplessness and hypochondria, it seemed to calm down - somewhat similar to the car battery, as long as it is full. In 1922, a study reported that lithium is not suitable to gelatinize banana extract (experts recommended pectin instead). A first clinical trial studying effects of lithium in ten NPCD patients was published in 2021 by the same team showing some improvement of swallowing function after 12 months of treatment. Other neurologic parameters remained unaffected though. The new study used transgenic mice bearing the I1061T variant. The results show a mild prolongation of life span and a reduced loss of Purkinje cells, but no clear improvement of neurologic symptoms. Lithium influences many processes in cells, the study hints to changes in signaling pathways that have already been discussed in relation with NPCD (for pros: STING and SREBP2). Overall, however, the data obtained in mice seem to dampen a bit the enthusiasm for lithium as treatment.

https://pubmed.ncbi.nlm.nih.gov/37458497/
Mankind, as far as one can know it, seems to be divided in those who trash everything, and those who keep everything ("Never know what it's good for!"). Support for the latter party comes from a study from Japan (Rakib et al., 2023 Animals). The colleagues have reopened "old" cases of the 1980s. The suspects are cats, a Siamese and a Japanese cat breed. Luckily, someone kept the samples allowing the colleagues to sequence the genes and to identify the variant. The result is surprising. In both cats, the culprits are variants of NPC2 – rare and precious. The question is now whether there are more NPC2 variants lingering in Japanese or – more widely – Asian cat populations. They could help to establish another cat and thus large animal model for NPCD. As reminder, apart from cats, the only large animal model is angus cattle, that's probably as large as it can get.

https://pubmed.ncbi.nlm.nih.gov/37371089/
Next topic, gene therapy. How this works principally is probably known by now, several studies during the last years showed that a virus particle-based gene therapy works in NPC1-deficient mice. Virus particle-based, because here viral particles, notably the notorious adeno-associated virus (AAV), are used as vehicle to shuttle the normal NPC1 gene in cells. Ok, that sounds nice and easy, but the devil is in the detail imposing some fiddling. This is nicely illustrated by a study from the UK (Hughes et al., 2023 Cells). What's the problem? Well, it's very simple: the virus can carry around 4.7

kBp – no, not kilobtye, but kilobasepairs. Sounds heavy, but no. As reminder, biology class, the genome consists of base pairs (for example adenine on one string of the double helix that goes with thymine on the other etc.). Now, imagine each basepair as letter, and the virus as bottle. The AAV bottle can hold a letter with maximally 4,700 letters. That's approximately 1.6 pages of this doc. The protein-coding sequence of NPC1 is 3,800 letters long. "That's shorter, so what!", one may argue. Well, for gene therapy to work, a socalled promoter sequence is also needed. That's a stretch of DNA often in front of the gene that determines how much messenger RNA (and ultimately protein) is produced in which cell under whatever condition. This corresponds to – ok, the comparison hobbles – the address and the salutation. They determine in a way who reads the letter at which level of attention title ("Dear Madam, Dear Sir", "My dear", or "Juhuuuuuuuuu sugar babe" etc.). The promoter should be long enough to comprise necessary information, but short enough to fit in the virus and to resist breaks. By a quasi-herculean effort, the colleagues identified a relatively short promoter sequence preceeding the human NPC1 gene. This fragment helps cells in the brain to produce large amounts of NPC1. NPC1-deficient mice treated with this promoter plus the NPC1 coding sequence showed no tremor and survived three times longer than their untreated littermates. Stay tuned!

https://pubmed.ncbi.nlm.nih.gov/37407594/

Next topic, cable isolation. Nerve cells are cabled through specialized processes, axons and dendrites. Synapses can be considered as high-tech lustre connectors. Axons are wrapped by insulating layers called myelin enabling rapid and energy-efficient conduction of electrical signals. In the brain, this insulation is formed by oligodendrocytes. These cells form few (= oligo) branches (dendro) that wrap repeatedly around axons. This creates a mille-feuille-like structure, as lipid-rich as the puff pastry version. Btw, this is the reason why most cholesterol in the brain is contained in myelin. So what now? It's known since quite some time that myelin is defect in the brain of NPCD patients and animal models. Earlier studies hinted to oligodendrocytes as culprits, but what exactly goes wrong is unclear. The Lieberman troupe reveals new insight, their article appeared in a respected journal of the NATURE empire (Kunkel et al., 2023 Nat Commun). The colleagues studied what happens in the forebrain of NPC1-deficient mice during the first weeks after birth. During this time, most myelin is being formed. Among others, they used a meanwhile well established approach *single cell transcriptomics*, where nearly all messenger RNAs present in a cell are sequenced and analysed using advanced bioinformatics. This teaches which

proteins are produced by any cell at time point x. Curiously, the method also allows to identify each cell type based on the genetic fingerprint, nerve cells produce a distinct set of proteins compared to oligodendrocytes. Back to the topic: The results show that the oligodendrocytes fail to develop correctly from their precursors, and that many of the latter die. This may cause hypo- (meaning under-) myelination. The story goes on, but it gets complicated. To make it simple, something goes wrong with the control of gene expression, more specifically the control by DNA-binding histones. Histones had already gigs on the NPCD stage in the context of a potential treatment based on histone-deacetylase inhibitors. One question raised by the new work is whether the specific defect in oligodendrocyte precursor cells can somehow be corrected.

https://pubmed.ncbi.nlm.nih.gov/37507375/
Thematically, we stick to the brain and turn to the million-dollar question why NPC1-deficient neurons die. There are many ideas, a new one is presented by the Dickson team in a tour-de-force article (Caras et al. 2023 Nat Commun). The new work follows up on previous studies by the group (see issue 6). The topic is once again calcium. Using an impressive arsenal of methods and a myriad of experiments (that's what it takes to get into NATURE empire) the team reveals an entire cascade of changes in "cultivated" neurons (nope, no opera, no theatre, but cell culture!) that causes inundation of mitochondria (energy!) with calcium and ultimately cell death. Steps in between are accumulation of specific calcium channels in the membrane surrounding cells, and more membrane contact sites inside cells, where specific organelles get in touch. The team also points to the trigger, possibly overactivation of mTORC, a main metabolic switch, and cyclic kinase 5. Interestingly, the study shows that inhibition of several intermediate steps can prevent the deleterious calcium flush of mitochondria. These steps may serve as therapeutic targets, their relevance has to be studied now in the brains of living animals.

https://pubmed.ncbi.nlm.nih.gov/37620691/
A team from Kumamoto in Japan sticks with beta-cyclodextrin. Continuing their previous work, the authors tested in cells and animals the efficacy of 9 different incarnations of cyclo with distinct diameters and chemical modifications (Yamada et al., 2023 Clin Transl Med). The results show among others that the damage to sensory cells in the ear occurs only with cyclos that are large enough to accomodate cholesterol.

https://pubmed.ncbi.nlm.nih.gov/37314654/
Time for some glamour in the NPCD field. It's about a real star in the neuroscience business, the socalled *brain-derived neurotrophic factor* or BDNF. PubMed lists 30,000 (!) scientific articles related to this small protein that was discovered in the 1980s in a Max-Planck Institute close to Munich (Germany). BDNF is a sort of factotum: it keeps neurons alive, regulates their development and guides them to their final place of work in the brain. BDNF also mediates processes in the adult brain such as memory formation, and it contributes to diseases such as depression. A first hint that BDNF-dependent signaling in NPCD is disturbed came up at the turn of the millenium based on experiments with cultured neurons. Since then, there was radio silence. New work from Italian colleagues (Lucarelli et al., 2023 Mol Neurobiol) shows that the BDNF system in the cerebellum of NPCD mice is broken, notably already during the first weeks after birth bevor the mice show neurologic symptoms. So, can BDNF prevent loss of neurons?

https://pubmed.ncbi.nlm.nih.gov/37369603/
An old, but at the same time new therapeutic approach was tested extensively by Japanese colleagues using NPC1-deficient mice (Yasuda et al., 2023 Life Sci Alliance). Old, because something similar had been tested before, new because other strategies have now been explored. The prime question is whether specific cells of the immune cells can prevent the loss of neurons. The colleagues attacked this by various tactics. To name a few: injection of bone marrow-derived cells into the abdominal cavity (intraperitoneal) saved nerve cells from death, but did not improve movement symptoms. Better effects were observed following intravenous injections of specific types of T lymphocytes from healthy donor mice. Within three weeks, ataxia was diminished and more Purkinje cells survived. Correspondingly, the mice got worse when they were deplete from specific lymphocytes by genetic or pharmacologic tricks. And they got better, if socalled inflammatory monocytes, a subtype of white blood cells, were removed. Further work has to show the robustness of these effects and their relevance in humans.

https://pubmed.ncbi.nlm.nih.gov/37024473/
Studies of NPC2, the small partner of NPC1, often appear under "Miscellaneous". A team from Denmark characterized a mouse model for NPC2 deficiency (Rasmussen et al., 2023 Mol Cell Neurosci). There is a whole gang of mice with defective NPC1, but the situation for NPC2 is rather poor. The examined mouse was established many years ago

by a company named BayGenomics, and it was used in previous studies. The new work delivers a more detailed description. The results can be summarized rather briefly. Onset and course of neurologic and visceral symptomes in NPC2-deficient mice are similar to those provoked by NPC1 deficiency. The mice live for at least 12 weeks. Taken together, the model can be used to study the consequences of defective NPC2 and possible therapies.

Animal models (ASMD)

https://pubmed.ncbi.nlm.nih.gov/37024473/

Finally, a study providing new insight in neurologic forms of ASMD (Gaudioso et al., 2023 Cell Death Dis). Let's start simple: defects in ASM cause accumulation of sphingomyelin. So far so good. But, sphingomyelin isn't sphingomyelin! Who'd have thought! There are different forms depending on the fatty acids that are built in. For example, palmitic acid (made from palm oil; attention readers fluid in ancient greek: hexadecanoate) has 16 carbon atoms, whereas stearic acid has two carbons more (octadecanoate). Where these distinct sphingomyelins are located within cells and what each of them does, is not clear. The Ledesma troupe studied exactly which sphingomyelins accumuate in the brain of ASM-deficient mice. They report that sphingomyelin 16:0 (quizz: which one is it? right, hexadecanoate) accumulates more strongly than others. Subsequent experiments showed that this very version of sphingomyelin kills nerve cells in culture. This raised the obvious question: Can this be prevented? Maybe! The enzyme *ceramide synthase 5* produces preferentially the deleterious version of sphingomyelin. Reducing the activity of this enzyme in the brain of ASM-deficient mice by a genetic trick reduced death of nerve cells at least a bit. Moreover, the specific sphingomyelin could be detected in the blood of mice with its concentration correlating with disease progress. So, sphingomyelin 16:0 may serve as new therapeutic target and biomarker.

https://pubmed.ncbi.nlm.nih.gov/37298714/

And here's another study from the Ledesma factory (Gaudioso et al., 2003 Int J Mol Sci), this one with the motto "Tried it, didn't work". The colleagues followed up on the legitimate assumption that a reduced dietary intake of choline may lessen symptoms in ASMD, notably for the neurovisceral forms. Why that? Choline is an important component of many substances in our body including the neurotransmitter acetylcholine, but also lipids like sphingomyelin. Our cells can produce choline, but this is complicated, and so food is used as preferential source. To test their assumption, the

colleagues fed ASM-deficient mice a cholin-free diet starting at eight weeks of age, when neurodegeneration kicks in. Unfortunately, neither the accumulation of sphingomyelin in liver or brain nor the loss of neurons were reduced. But it was definitely worth a try, and the authors should be lauded for the publication of negative results. This isn't really pleasant, but very important for the field.

https://pubmed.ncbi.nlm.nih.gov/37341882/
Studies with olipudase alfa show that enzyme replacement works. But, a major hurdle remains: the blood-brain barrier. This issue is addressed by a new study from Spain (Loeck et al., 2023 Drug Deliv Transl Res), which further explores a kind of piggypack system to deliver the enzyme to the brain. As reminder, the barrier is formed by endothelial cells that line the interior of blood vessels and batten down the hatches. One possibility to overcome the hurdle are socalled nanocarriers, specific (small) materials that can be charged with enzyme to carry the load into the brain. To this end, these are coupled with proteins that are normally taken up by endothelial cells. One example is transferrin, which – yes! – ferries iron into the brain. Another example are antibodies against a protein named ICAM that sits on the surfance of the cells. The idea to use nanocarriers isn't new, it was already tested starting in the 2000s in mice. The new results reveal an important aspect: defect in ASM activity modifies the efficacy of several of these carriers because it changes the target proteins on endothelial cells. In this context, ICAM remains a valid target as its presence on endothelial cells is increased in ASM-deficient mice. More protein, better piggyback!

https://pubmed.ncbi.nlm.nih.gov/37633404/
We stay with the topic nano-mule although with a distinct principle of operation. A team from Michigan (USA) explored a therapeutic approach for ASMD (Halseth et al., 2023 Nanomedicine) that was originally developed for cardiovascular disease. Ok, one more time – but you can also skip the next paragrah:
Supposedly, there is good and bad cholesterol.This is pure nonsense. Cholesterol is always the same, it depends on the wrapping. So, we got LDLs and HDLs in the blood (see your last blood check), which stand for *low* and *high density lipoproteins*, respectively. These are tiny, nanometer sized fat droplets with a protein coat. The LDLs transport lipids to cells in different organs, the HDLs transport superfluous lipids back to the liver for storage or excretion as bile. Yes, it's more complicated, and no, neither LDLs nor HDLs overcome the blood-brain barrier. There are several diseases, where the LDL level is too high and/or the HDL level is too low and where lipids accumulate in

tissues. This includes ASMD. Now the idea: produce artificial HDL particles that after administration free cells from unwanted lipids. The main element of these particles is a protein called apolipoprotein A1, which docks to a transporter in the cell membrane and takes over lipids. A fragment of ApoA1 is bound to a synthetic carrier, and voila, you got your HDL-like construct. So much for the theory.
The present study in ASMD-deficient mice brings first, but tender hints that the approach may work with limits. ASMD mice that received the synthetic HDL for three weeks intraperitoneal (in the belly) injections showed improved liver function – that's it. The liver remained enlarged and the neurologic symptoms did not improve. It remains to be seen where this approach will go.

Animal models (ASMD and NPCD)
https://pubmed.ncbi.nlm.nih.gov/37121555/
All good things are threes (or more!), another study from the Ledesma assembly line (Soto-Huelin et al., 2023 Neurobiol Dis), the topic is a new therapeutic approach for both diseases. The starting point is the previously mentioned idea that cells can spit out accumulated lipids. One way is the release of socalled extracellular vesicles, these are very much *en vogue*, a hot topic (see issue 6). The colleagues studied whether specific drugs induce this release and thereby revert pathologic changes in cell and mouse models of ASMD and NPCD. One of these molecules is called ellagic acid. It's a natural substance from plants, present in many fruits including black-, straw- and raspberries, pomegranate, nuts and – attention – all sorts of wines as long as they were stored in oak barrels. The molecule is converted by gut bacteria to another molecule named urolithin, which seems to be the active substance. The comprehensive study shows that treatment of ASM- or NPC1-deficient cells increases the release of extracellular vesicles and decreases the accumulation of respective lipids. Oral administration of ellagic acid for three months improved slightly motor control, weight gain and reduced sphingomyelin accumulation and lessened microglia activation in ASM-deficient mice. Similarly positive effects were seen in NPC1-deficient mice. Curious, how this will evolve.

Cell-based models (NPCD)
https://pubmed.ncbi.nlm.nih.gov/37440478/
"Good fishing!" A study from New Zealand is about fishing, promising waters and the right bait (Hammond et al., 2023 Genetics). What is fished? Proteins that bind to NPC1. Where's the angling? In yeast, the favourite fungus of biomedical research. What's the bait? Parts of the NPC1 protein, here the yeast version, named *Niemann-Pick type C-*

related protein-1 or Ncr1. This version is able to replace human NPC1, although it probably has diverse functions in the unicellular being. Several groups have undertaken similar campains in yeast and other cells, the catch was often small but precious. The principle is similar, take each protein produced by yeast and test whether it takes the bait (binds to NPC1). Admittedly, it's more complex and certainly time-consuming. For example, you need an alarm indicating when fish bites. Three new and surprising interaction partners of NPC1 were caught, future studies will show, which ones are disease-relevant.

Cell-based models (ASMD)

https://pubmed.ncbi.nlm.nih.gov/37628828/

New experimental models are essential to advance preclinical research. As mentioned in a previous issue (number 4), there is a hype about socalled organoids. In principle, this is a kind of Bonsai version of the organ of interest, well kind of. Organoids are formed in test tubes by reprogrammed cells from patients. A Spanish group reports the generation of organoids from a liver biopsy of a patient with chronic-visceral ASMD (Niemann-Pick type B) (Gomez-Mariano et al., 2023 Int J Mol Med). The patient carried the relatively frequent Arg610del mutation. The results show that the organoids recapitulate many features of patient livers. Therefore, they may serve as model for future studies. A challenge is the generation of a "control organoid". A simple, but non-optimal solution are organoids derived from healthy donors. However, these are genetically different. Ideally, the controls are "isogenic", meaning that all genes are the same except of course for the pathogenic mutations in SMPD1 (the ASM encoding enzyme). This can be done nowadays, but it's more work and more costly. In any case, this is just the beginning, there will be more to come.

Miscellaneous

https://pubmed.ncbi.nlm.nih.gov/37443907/

Once again, seafood lovers attention please. A study from China shows: feeding the white leg shrimp (*Litopenaeus vannamei*), an important aquaculture animal, a bile acid from goose enhances the amount of NPC1 in shrimp, and improves their growth and lipid metabolism (Shi et al., 2023 Animals). For humans, the detour through the shrimp is probably more palatable than direct intake of the bile acid.

https://pubmed.ncbi.nlm.nih.gov/37602789/
An excursion to agricultural pests, the tiny silverleave whitefly (*Bemisia tabaci*), a feared enemy of farming and ornamental plant growers. A Chinese-Ukrainian-American team showed that the animal has only one NPC1 protein (Yu et al. 2023 Arch Insect Biochem Physiol). This is surprising, because most insects have two. Reduction of NPC1 using a trick renders flies sterile and sick causing their premature death. So, once again NPC1 proteins may serve as target for pest control.

https://pubmed.ncbi.nlm.nih.gov/37423302/
Romanian colleagues report surprising insight with respect to NPC1 and melanin (Rus et al., 2023 J Biol Chem) – not to be confused with melatonin! Melanin is the stuff that together with other factors determines the color of eyes, hair, and skin. It protects from UV radiation, and it may have other functions in the immune system. Melanin is produced by special cells named melanocytes, where it is stored in specific structures called melanosomes. The team showed in melanoma (= tumor) cell lines that deficiency of NPC1 perturbs the production and storage of melanin. How exactly is unclear. There are changes in the different proteins that are required to produce and distribute the stuff. Are there pigment changes in NPC1 patients? Possibly, in the socalled pigment epithelium of the eye, which produces melanin.