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Dear Readers.
We keep going! Welcome to the third edition of Pfrieger's Digest covering the period of August 1 to October 31, 2020. Here's the link for the PubMed search:

((niemann pick type c OR niemann pick type C1 OR niemann pick type c2 OR npc1 OR npc2) AND (("2020/08/01"[Date - Publication] : "2020/10/31"[Date - Publication])) NOT (("2020/01/01"[Date - Publication] : "2020/07/31"[Date - Publication])).

During this period, **48** articles have been published in scientific journals. As already mentioned: 1) Review articles or case studies do not receive comments. 2) I only describe articles that I can read in full, 3) the selection is subjective, 4) I try to make sure that the information is correct, but I cannot guarantee that, 5) Ratings and interpretations are my personal opinion and I make no claims of validity.

Patients
https://pubmed.ncbi.nlm.nih.gov/32073546/
Solomon et al. (2020) examined the influence of Miglustat/Zavesca on swallowing disorders and aspiration risk, which means the risk of choking. It's hard to believe, but swallowing is a highly complex process that is partially controlled voluntarily and partially by reflexes—neuronally anyway. Previous studies of NPC patients showed that miglustat slowed the worsening of swallowing problems. The new study on 50 patients confirms this by and large with further quantitative data (numbers, that is) from standardised tests.

https://pubmed.ncbi.nlm.nih.gov/32931663/
Silent night, holy night—no, almost! A Portuguese study (Encaranaco et al., 2020) addresses so-called *silent* gene variants—that is variants of the NPC1 gene that were thought to be harmless. At least we thought so! The study examined patients with NPC symptoms, whose molecular analyses did not reveal a clear picture. The study found that some of the patients had a silent variant, called p.V562V, and that this variant was not at all that harmless. It generates a shrunken protein and a blueprint, i.e. the messenger RNA, that ends up in the cellular rubbish bin. At the same time, some cells can iron out this error and produce completely normal NPC1. Which cell does what is still unknown. In any case, the results explain that the filipin test with fibroblasts in said patients showed neither the classically strong nor *variant* cholesterol accumulation, but something in between. So, remember: not every silent variant is silent, just as not every silent night is holy.

https://pubmed.ncbi.nlm.nih.gov/32993765/
Speaking of night: a study from Japan (Imanhishi et al., 2020) is about sleep and cataplexy in NPC patients and hypocretin, also called orexin. What do they have to do with one another? Hypocretin is a peptide hormone that is made in the hypothalamus. It regulates all sorts of things, including sleep. The study confirms previous observations that NPC patients with cataplectic seizures have low levels of hypocretin in their cerebrospinal fluid. Incidentally, massive defects in this hormonal system lead to narcolepsy, a bizarre sleep disorder often associated with cataplectic attacks. This can have genetic causes: Studies of narcoleptic dogs—an animal model—led to the discovery of a genetic defect in the hypocretin receptor, a very interesting story but off topic here. Alternative causes may be autoimmune attacks against the hypocretin-producing nerve cells in the hypothalamus. Cut a long story short: Cataplexy in NPC patients could be caused by hypocretin deficiency. This in turn may be genetic, or caused by the degeneration of corresponding nerve cells.

Animal models
https://pubmed.ncbi.nlm.nih.gov/32770132/
News from the gene therapy front? The employees of a company tried an alternative to virus-based gene therapy (Jiang et al., 2020). cDNA, which contains the blueprint for NPC1, was packed into a sort of artificial fat droplet or liposome. These were then additionally decorated on the outside with an antibody that binds to the so-called transferrin receptor. The latter serves to facilitate uptake of the droplets by cells, and thus facilitates entry of the DNA. It also helps to overcome the blood-brain barrier. Devil knows why this vehicle is called *Trojan horse*. Anyone familiar with Homer will notice that the name is rather unfortunate—I spare the details here. In any case, liposomes were then administered to six-weeks-old NPC1-deficient mice by injection into the bloodstream. So far so good. Unfortunately, the study disappoints a bit in terms of content. There are virtually no quantitative data describing the effects on the mice.

https://pubmed.ncbi.nlm.nih.gov/33204596/
A study by Chen and colleagues is about blood, or more specifically about platelets. These cells, which are not *real* cells because they lack nuclei, are among other things, responsible for blood coagulation and thus for wound closure. The study shows that NPC1-deficient mice have around 30% more platelets, but that they clump less well than normal ones. Consequently, the mice show prolonged bleeding after injury. And how about patients? Well, big difference, because the number of platelets was in the normal range, even tending towards the lower boundary. So, another example of how mice and men are different.

https://pubmed.ncbi.nlm.nih.gov/32970694/

First, a bit of self-promotion: we have just published an open access article dealing with experimental models for research on NPC (Pallottini & Pfrieger, 2020). In a first draft, we wrote that there are no models using large animals, i.e. animals larger than cats. We thought so! Promptly, a study appeared on our screens that refuted this statement (Wooley et al., 2020). A group from Australia actually found NPC1 mutations in a commercial herd of Aberdeen Angus cattle. Several calves developed movement disorders beginning at the age of three months. They walked sideways and fell over. Some died prematurely—not by slaughter. Food poisoning, for example by *Swainsona formosa*, also called *famous flower* (!?) was excluded. Further investigations showed that the animals bear a new variant of NPC1. So there is a large animal model for NPC—even bigger than expected! It will be interesting to see what this will be good for.

https://pubmed.ncbi.nlm.nih.gov/33016621/

An interesting study by Bartoll and colleagues is actually about Niemann-Pick type A, but there is also data on type C. The group shows the first preclinical data for a new therapeutic approach based on cannabis. No—watch out! More precisely, the approach is based on the brain's own endocannabanoid system. Endocannabinoids such as anandamide are fat-like substances. These are synthesized by brain cells and act as so-called neuromodulators. They control a multitude of processes, including learning and memory, and play a role in certain neurological and psychiatric diseases. Similar to classic neurotransmitters, the endocannabinoids activate corresponding receptors. There is one important difference, however. The endocannabinoids act within membranes, they do not bind from outside. The Ledesma group found that in nerve cells from ASM-deficient mice, a model for Niemann-Pick type A, the endocannabinoid receptor type 1 is virtually lost, probably due to pathological accumulation of sphingomyelin. This in turn disrupts signal transmission based on it. Now, of course, you could administrate cannabinoids artificially, for example by smoking. However, that would definitely be the wrong way due to side effects. An alternative method is to inhibit the brain's own breakdown of endocannabinoids. This is done by certain enzymes with terrifying names like *fatty acid amide hydrolase* and *monoacylglycerol lipase.* Treatment of ASM-deficient mice with substances that inhibit these enzymes reduced the accumulation of sphingomyelin, possibly by activating the neutral sphingomyelinase. Treatment also reduced movement disturbance and prolonged the lifespans of the mice. Positive effects were also found in NPC1-deficient mice, but the focus of this work was on Niemann-Pick type A. The endocannabinoid system may be a worthwhile target for therapeutic approaches for both diseases.

https://pubmed.ncbi.nlm.nih.gov/32931479/
We stay with potential therapeutic targets. A study by Liu et al. brings a new candidate into play, known as lysophagia. This process belongs to the cell's quality control system, which removes broken proteins and other components. In this case, it *eats* the lysosomes, the notorious setting for the cellular NPC drama: the pathological accumulation of cholesterol and other lipids. The Lieberman group first asked whether lysophagia still works in cells with broken NPC1: Yes! Damaged lysosomes are disposed of. Then they investigated whether and what happens when one disturbs the lysophagia. NPC1-deficient mice were crossed with mice that lack a certain component of the complicated lysosome disposal machinery, the so-called Fbox2 protein. Bingo! The double-deficient mice died even sooner, and showed greater damage in the cerebellum than *normal* NPC1 mice. Conversely, this means that overactivation of lysophagia could possibly be used therapeutically if there were appropriate activators.

https://pubmed.ncbi.nlm.nih.gov/33163944/
Another possible therapeutic target, the APL1 or c/Abl protein, is the subject of a new study from Chile (Contreras et al., 2020). The abbreviation sounds puzzling. It goes back to a virus that is named after Mr Abelson and that has inserted the corresponding gene in its blueprint. ABL1, a protein kinase, is one of the central switches in the cell that controls many processes, including division, differentiation, and also programmed death. This kinase plays a central role in a certain form of leukaemia, and drugs that inhibit the kinase, including imatinib, are now used successfully to treat patients. By the way, chemicals or other substances that are turned into therapeutic drugs frequently change their names, especially after company takeovers and as a marketing action: Originally, the substance was called CGP57148B, developed by Ciba Pharmaceuticals in Basel, Switzerland, hence the first letters for Ciba Geigy Product. Then it was baptized STI-571, then imatinib, and finally Glivec or Gleevec, an FDA-approved blockbuster, whose patent protection has expired. Back to NPC: Silvana Zanlungo's group had already shown a few years ago that imatinib slows down neurodegeneration in NPC1-deficient mice and expands their lifespan to some extent. The new study confirms these results, showing that the effect may be based on a disinhibition of lysosome production.

https://pubmed.ncbi.nlm.nih.gov/33079236/
So long, and thanks for all the fish! No, we're not there yet, but it's about fish, more precisely zebrafish or *Danio rerio.* This freshwater fish, native to Asia, is a popular object of biological research—and probably also of aquarists—, and serves as a model for research on various human diseases. The fish is relatively small—imagine using a giant catfish instead—and easy to breed. The embryos are transparent and develop relatively quickly. You can virtually watch them grow up. NPC1-deficient

zebrafish have already been presented in earlier studies. They initially develop normally, but then show pronounced defects that are quite similar to human symptoms, including motor changes, balance disorders and premature death. The latest study presents a pharmacological fish model, where the NPC1 inhibitor U18666A is added to the aquarium water (Cook et al., 2020). The group shows impressive images of fat accumulation in the whole animal obtained by *light sheet microscopy*, where a new kind of sheet-like illumination generates high-resolution, three-dimensional images of relatively large, even living, objects, such as fish larvae.

Cells

https://pubmed.ncbi.nlm.nih.gov/33081384/

A new study deals with nerve cells obtained from so-called induced pluripotent stem cells (Juers et al., 2020). These stem cells are shaking up biomedical research. The starting point is, among other things, the well-known fibroblasts, which are usually taken from patients' skin. These cells are reprogrammed *in vitro*, i.e. in cell culture, by inserting certain genes, so-called transcription factors, into them. These factors switch on (or off) expression of entire groups of genes, and thus enable the conversion of old fibroblasts to new pluripotent stem cells. They are called *pluripotent* because they can do a lot—but not everything, otherwise they would be *omnipotent*. In further steps, these cells can be used to make highly specialised cells, including nerve cells. Because these cells come from patients, their properties are, of course, more suitable for studying the causes of disease and testing therapies than those from animal models. This area is developing rapidly, last not least because *teething troubles* of the model, such as lacking standardisation of experimental protocols and variability in cell yield and quality (purity, survival) are being brought under control. By the way, it typically takes a few weeks to transform cells from a scrap of skin into nerve cells. The Frech group and other teams around the world have been studying nerve cells from NPC patient fibroblasts for some time. The latest study deals with *oxidative stress*. This process is caused by highly reactive oxygen compounds that can badly devastate cells and that are usually kept in check. There is increasing evidence that this much studied *phenomenon* plays a role in NPC, but the results are partially contradictory. Watch out! Cell cultures, for example, are confronted with atmospheric oxygen at 21%, which does not reflect oxygen concentrations in living tissues. In addition, the question of proper *controls* generally arises with pluripotent stem cells. Many studies use stem cells from healthy donors. But these cells are genetically and even epigenetically completely different, because every person is different. If one only wants to examine the influence of the *NPC1* mutation, one would have to produce so-called *isogenic* control cells. To accomplish this, you have to genetically reverse the NPC1 mutation in the cells of a particular patient. This is possible, but laborious.

Miscellaneous

https://pubmed.ncbi.nlm.nih.gov/33087814/

A message for bird keepers and poultry farmers: different variants of the NPC2 protein have been discovered in the olfactory antennae of the red bird mite (*Dermanyssus gallinae*), a nasty pest that sucks blood from birds. What NPC2 does there is still unknown.