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Dear Readers.

Welcome to the fourth issue of Pfrieger's Digest. You will notice incremental changes in the design: a portrait of Ludwig Pick ornaments the header. However, the image of Albert Niemann is missing, I could not find any. Therefore, my call for a world-wide campaign:

Finding Niemann

Maybe somebody can find a photo, or draw or paint a fantasy portrait. Photos on the WWW show his father, an opera singer, or the famous chemist, both bearing the same name. I would love to receive your suggestions and put them in the placeholder. So much for that!

The new issue covers the period from November 1st 2020 to March 15th 2021. The link for the corresponding PubMed query is:

((niemann pick type c OR niemann pick type C1 OR niemann pick type c2 OR npc1 OR npc2) AND (("2020/11/01"[Date - Publication] : "2021/03/15"[Date - Publication])) NOT (("2020/01/01"[Date - Publication] : "2020/10/31"[Date - Publication])).

In this period, **54** publications were published in scientific journals. I would like to note that a considerable number of publications lists authors' conflicts of interest (for example because an author is employed by a company or because an author received a honorary etc.). Most journals demand this declaration. Please note further: 1) I describe articles where I have full-text access. 2) My selection is absolutely subjective. 3) Review articles or case studies are not commented. 4) I try to ensure correctness of statements, but I cannot guarantee this. 5) Judgements and interpretations reflect my personal opinion and do not claim any validity. 6) I apologize for all errors (typos, orthography, grammar, expression).

Please feel free to forward this issue. Feedback is welcome to fw-pfrieger@gmx.de or frank.pfrieger@unistra.fr.

Patients

https://pubmed.ncbi.nlm.nih.gov/33139814/

Chiorean et al. (2020) studied the type and frequency of NPC1 mutations using the "Genome Aggregation database" or gnomAD. This collection contains genetic information (for specialists: exome and genome sequences) of 141.456 non-related individuals. Originally, the authors asked whether heterozygous carriers of NPC1 mutations have a higher risk of obesity. Some previous studies seemed to have uncovered such connection, others did not probably depending on the dataset and the definition of obesity. In any case, the new study reveals that the frequency of NPC1 mutations varies across ethnic groups. A key question is how representative such collections are given the 10 billion or so humans on Earth and how meaningful the attribution to ethnic groups.

https://pubmed.ncbi.nlm.nih.gov/33257258/

Sidhu et al. (2020) introduce a new blood test that reinforces the "biomarker" team. Remember: biomarkers are substances, ideally detectable in blood, that allow to diagnose NPC and to assess the success of therapeutic approaches. Meanwhile, there are three players on the field, the oxysterols (c-triol), the oxysterol-derived bile acids including the so-called TCG [i.e. N-(3β,5α,6β-trihydroxy-cholan-24-oyl)glycine] and N-palmitoyl-O-phosphocholine a.k.a. LysoSM-509. The new test is based on TCG and seems to detect Niemann-Pick type C patients more reliably than the other players. We shall see how it performs under real-world conditions.

https://pubmed.ncbi.nlm.nih.gov/33228797/

Mengel et al. (2020) report results of a prospective clinical study sponsored by Orphazyme. They describe the disease progression in 31 patients during periods ranging between 6 to 14 months using a simplified version of the NPC Clinical Severity Scale and different biomarkers. All patients received Miglustat/Zavesca, half of the participants presented disease onset at late infantile stage. What do we learn? The disease progressions are highly variable and do not correlate with the types of mutations. The simplified scales seems more or less as good as the more complicated version. With respect to biomarkers, oxysterols correlated relatively well with the severity scale. Evidently the same caveat applies: the relatively small number of patients together with the large variability between patients make it difficult to draw conclusions. It will be interesting to see the Arimoclomol data, in particular a comparison of symptoms and disease progress before/after the treatment.

Animal models

https://pubmed.ncbi.nlm.nih.gov/33345999/

Meneses-Salas and colleagues (2021) present a new episode of the series "It's not what you think, honey!". Previously different teams showed that a specific protein named annexin-6 plays a role in cholesterol transport within cells. Higher amounts of annexin-6 caused cholesterol accumulation similar to NPC1 dysfunction. Another study in cell cultures showed that lowering the amount of annexin-6 reverts cholesterol accumulation in NPC1-deficient cells. Great! But no: against all expections, things are different in living animals: genetic elimination of annexin-6 in NPC1-deficient mice aggravated liver disease and shortened the life span. Thus, the study *in vivo* rendered the opposite of what was expected based on cell culture experiments. Welcome to biology!

https://pubmed.ncbi.nlm.nih.gov/33738443/

News about acetylleucine, also known as Tanganil: the teams of Michael Strupp and Frances Platt (Kaya et al., 2021) report about its effects in NPC patients and mice. As a reminder: mice of the BALB/c strain lacking NPC1 develop first neurologic symptoms during the sixth week of life and die at 10 to 12 weeks of age. Healthy mice reach two years of age. The study shows that acetylleucin improves motor control in patients and in mice. The latter even show slightly longer life spans, when treated with a combination of acetylleucine and Miglustat/Zavesca. With respect to the mode of action, the mouse data suggest that acetylleucine interferes with sugar metabolism and oxidative stress. But now, watch out: the study shows that only the L, but not the D form of acetylleucine is active in mice. What' this all about? Some chemistry: many molecules, including amino acids like leucine, exist in two, sort of mirrored forms, named D and L, a bit like left and right hand. Chemists call this chirality. Now, the important thing is: the D and L forms of a molecule often have completely different biological effects. Classic example noticed first by the Italian chemist Piutti by the end of the 19th century: the amino acid L-asparagine (asparagus!) tastes sweet, D-asparagin doesn't. An earlier study by Strupp and Platt indicated that acetyl-D-leucine probably inhibits uptake of acetyl-L-Leucine or its conversion to L-leucine. Tanganil is a 50:50 mix of acetyl-L-leucine und acetyl-D-leucine, chemists call this a racemate. Based on the new data it appears that this isn't optimal, because first you only get half the dose, and second the D form may impair the efficacy of the L form.

https://pubmed.ncbi.nlm.nih.gov/33466390/

The biomarker topic stays with us: a study from Japan (Fukaura et al., 2021) shows that intracerebroventricular (meaning directly into the brain) injections of 2-

hydroxpropyl-beta-cyclodextrin in NPC mice lowers the concentration of a protein named *glycoprotein nonmetastatic melanoma protein B* (it goes under other names, this is one I find the best). Previous studies showed increased blood levels of this protein in patients with NPC, Parkinson und Alzheimer and with specific types of cancer. The functions of the protein are not clear yet, it is present in many organs including the brain, where it may dampen inflammatory reactions. There's more to be uncovered!

https://pubmed.ncbi.nlm.nih.gov/33256498/

Colleagues from Japan report another successful attempt of gene therapy in NPC1-deficient mice (Kurokawa et al., 2021). Since 2017, three articles have been published showing the feasibility and efficacy of virus-based gene therapy in mice. The new study builds on the previous ones and brings a new trick that improved the outcome. What do we need for gene therapy? First, a so-called vector that carries the genetic plan of normal NPC1 protein to the cells. Like others previously the Japanes group used the adeno-associated virus 9 or AAV9. Second, we need a so-called promoter, a sequence in the DNA that determines where and when a gene is expressed and thus the desired protein (in our case NPC1) produced. In the present study, a "strong" promoter sequence was used. This one drives gene expression "full throttle" regardless of the type of cell. Third, the vector needs to reach the target cells, in our case neurons. Here, the Japanese colleagues used a simple trick: they injected the virus by two routes into the brain, in the lateral vehicle and in the cisterna magna. These double injections led to a stronger distribution of viral expression than single injections. The results are encouraging: once injected in NPC mice at the age of 5 to 6 days, their life span was doubled, the motor defects and the death of Purkinje cells in the cerebellum were strongly reduced. Evidently, there is still room for improvement.

https://pubmed.ncbi.nlm.nih.gov/33627648/

The group of Sabina Tahirovic in Munich (Colombo et al., 2021) studied so-called microglial cells in NPC1 mice. These cells act as national guard, fire department and garbage disposal at the same time. In NPC mice, the cells show defects that may contribute to the dysfunction and degeneration of nerve cells. Moreover, the cells may serve as target for new therapies. Indeed, these cells have been long neglected, because it was thought that nerve cells die simply because they lack NPC1. Don't count your chickens before...!

https://pubmed.ncbi.nlm.nih.gov/33722902/
An episode of "Dreams Come True". In our recent review (Pallottini & Pfrieger, 2020) we mentioned the need for more models based on NPC2 deficiency. Et voilà: there is a new fish model based on NPC2, two fish with NPC1 deficiency were already on the table (see PD issue 3). The group of Denny Porter (Tseng et al., 2021) shows how Zebrafisch (*Danio rerio*) are doing without NPC2: very badly! Cholesterol accumulates, motor control is impaired and the fish die prematurely. The whole thing gets worse, if the fish cannot get maternal NPC2. Remember, the protein is secreted and thus can spill over from fish mother to fish kid.

Cells

https://pubmed.ncbi.nlm.nih.gov/33144569/
Back to the topic "new players": Van den Boomen and colleagues (2020) report the addition of a new player to the big team of proteins that regulates the cholesterol level of cells. One could think that all is said and done after 250 years of research on this topic, namely that all players are known. Nope! The group - as others before - has performed a so-called genetic screen. This trick allows to identify components that contribute to normal or pathologic processes in cells. What do we need? First, a readout, some sort of measure that monitors the process of interest, and second a means to eliminate every single gene that is expressed in the cell. Imagine, you had a radio but no idea how this works. For the screen, you would listen to the music of your favorite station (readout), then remove each component of the radio (genes), and check each time, whether you can still hear the music. Van den Boomen used for their screen a cell line (the radio) and as readout (music) a light signal that reports whether the cell switched on its cholesterol-synthesizing machinery. To cut a long story short: a gene named *C18orf8*, whose function was unknown - therefore the name! - belongs to the big machine that determines whether an important cellular switch, the so-called Rab7 protein, is ON or OFF. This switch seems to regulate, whether and how cholesterol can be escorted out of the endosomal-lysosomal system via NPC1. Increase level of Rab7 reduced the accumulation of cholesterol in fibroblasts of NPC patients. There we go!

https://pubmed.ncbi.nlm.nih.gov/33308480/
We stay with the topic "switches". The team of Roberto Zoncu (Davis et al., 2021) studied what happens to the lysosomes of cells once the NPC1 protein is "kaputt". Just as reminder: the lysosomal is a sort of shredder and recycling center of the cell (the trash bin with the yellow lid in Germany). The group reports that deficient NPC1 impairs lysosomal protein shredding and the recycling of mitochondria, the cells power plants. Moreover, another important cellular switch, the so-called

mTor1, is permanently set to ON. The group further shows that blocking mTor1 can repair some of the cellular damage. Now, we shall see whether and how these observations are true in highly specialized cells of living organisms.

https://pubmed.ncbi.nlm.nih.gov/33311479/
Back to the topic "Dreams Come True". Pallottini & Pfrieger, 2020 also hoped that there will be experimental models based on so-called organoid cultures for NPC. Et voilà 2, instant delivery: A Korean group has just introduced such a model. What's this about? Normal cell cultures are two-dimensional: cells live on the flat surface of a plastic dish - like people in flatlands. That's quite useful, but it hasn't anything to do with reality in a living organism, where all organs, even skin, have a three-dimensional structure made of different kinds of specialized cells. This is exactly what organoid cultures aim to mimick: different cell types come together in a three-dimentional matrix and form a miniature organ (brain, liver etc.). Lee et al. (2020) produced brain organoids from NPC fibroblasts. This required their re-programming to so-called neural stem cells and then their differentiation to highly specialized neurons and non-neuronal cells. Therefore, production of organoids is more challenging than use of run-of-the-mill cultures. The results show that NPC organoids developed some characteristic features of NPC including the accumulation of cholesterol in the endosomal-lysosomal system. Moreover, preliminary experiments validate their use for drug tests. We shall see whether these new models gain followers.

Miscellaneous
https://pubmed.ncbi.nlm.nih.gov/33141558/
The team of Daniel Wüstner (Moesgaard et al., 2020) shows in test tubes that the yeast version of the NPC2 protein, the nimble helper of the big NPC1, binds and transports lipids other than cholesterol, notably the so-called phospholipids. Future experiments will show whether this is also true for "our" version of NPC2. It's possible that the binding properties of this protein have been refined during evolution (remember: yeast came before man) such that the mammalian version binds only cholesterol - or not.

https://pubmed.ncbi.nlm.nih.gov/33240109/
https://pubmed.ncbi.nlm.nih.gov/32750126/
https://pubmed.ncbi.nlm.nih.gov/33418888/
News from the animal kingdom, this time about insects and those rare velvet worms (Onychophora, for zoology freaks). *Arma chinensis*, a species of predatory stink bugs, is increasingly used for biological pest control. Chinese scientists show that the

animal bears NPC2 in its trunk-like olfactory organ to find prey and probably mates. If animals were starved, the amount of NPC2 in trunks of males (but not of females) decreased. Scientists from Spain and Germany showed that the two-inches-long velvet worm *Euperipatoides rowelli* living in the humid forests of South-East Australia bears a record 11 different NPC2 genes - more than the fruit fly! Last not least, the NPC2 gene is expressed in the alfalfa-pollinating bee *Megachile rotundata*, but unfortunately not stably enough to serve as biomarker reporting on the health of the bee. Good to know!

https://pubmed.ncbi.nlm.nih.gov/33191681/

Finally, news for pet friends and those who suffer from pet allergies: Chinese colleagues show that NPC2 is a cat allergen that provokes stronger reactions in women than men. This study confirms previous work revealing NPC2 as mite and dog allergen.