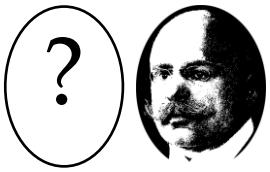
Pfrieger's Digest



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Niemann-Pick Type C

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Dear Readers.

Welcome back to Pfrieger's Digest – it took a bit longer. This new edition covers a relatively long period from March 16th to August 31st 2021. The link to the PubMed query is:

((niemann-pick type C disease OR niemann-pick disease type C OR niemann-pick type C1 OR niemann-pick type c2 OR npc1 OR npc2) AND (("2021/03/16"[Date - Publication] : "2021/08/31"[Date - Publication]))) NOT (("2020/01/01"[Date - Publication] : "2021/3/15"[Date - Publication]))

In this period, 84 articles were published in scientific journals including 8 reviews.

As always, the following statements apply: 1) My selection of articles is totally subjective, 2) I do not include review articles or case studies, 3) I only describe articles which I can read entirely, 4) I try to ensure correctness of statements, but I cannot guarantee this, 5) judgements and interpretations expressed in this document are subjective and reflect my personal opinion, they do not claim any validity, 6) I apologize for errors (grammar, orthography etc.). Please feel free to distribute this issue. Feedback welcome to fw-pfrieger@gmx.de or frank.pfrieger@unistra.fr.

Patients

https://pubmed.ncbi.nlm.nih.gov/34418116

The results of a clinical phase 2/3 study with arimoclomol (ARI) were published (Mengel et al., 2021). The study examined, whether ARI slows down disease progression compared to placebo. This was measured based on a simplified version of the NPC Clinical Severity Scale that was reduced to five domains. Overall, results were obtained from 42 patients, 16 of whom received the sugar pill and 25 ARI. Well, what can be said? ARI is not a magic potion. But there may have been a measurable slowdown of the worsening in some patients. One criterium to judge drug efficacy (which among other things conditions drug approval) is the socalled p value, which comes out of the statistical analysis. It's value at 0.046 for all patients is rather sobering. What does this mean? Well, if only we could know. If you ask scientists what the p value means exactly, you'll get lots of wrong answers - mine included. There is a big debate since decades how to decide whether differences between two groups (drug vs. placebo) are significant. The key problem with NPC and similar disease is the variability among patients, which makes statistical analyses inherently

difficult as values hardly obey laws, for example to follow a distribution named after good old Gauss. Two remarks: first, it would be great, if publications such as this would show (and analyse) the values of individual patients over time plus information about mutations and biomarkers per patient. All too often, the articles show only mean values and thus loose information. One could check, for example, whether patient A or patient B, who seemed to show an ARI-induced change had eventually a specific mutation or a specific value of a biomarker. There are methods to look into this (for example by *principal component analysis*) that take into account all parameters (age, mutations, markers, treatment etc.) and that test whether patients fall into specific groups. In biology, these methods become increasingly important, because increasing numbers of measures are taken at the same time. We start to realize that biological structures and processes can only be described by large sets of parameters. Evidently, the number of patients may be too small for this kind of analyses. Second, one wonders how one should design clinical studies with rare diseases and such variable presentations. This is certainly not a new thought! However, the failures of cyclodextrin and ARI in clinical trials are a kind of shot across the bow.

https://pubmed.ncbi.nlm.nih.gov/34096670

This study (Bremova-Ertl et al., 2021) deals with an old hat, disturbed eye movements in NPC patients. Examining over 70 NPC patients, the study brings new insight that is relevant for the diagnosis of NPC and for the monitoring of disease progression. The *vertical supranuclear gaze palsy* is a well known symptom of NPC. The new data show that eye movements should be carefully examed. Well, you may think: eye movements, that can't be too complicated. They are! The human eye uses all sorts of tricks to change for example the line of vision, and even to keep the retina "well looking". One trick are saccades, which are in principle fast (100 milliseconds long), voluntary and involuntary eye movements. All of this is controled by several neuronal centers in the brain stem, which degenerate in several diseases, including Parkinson. Why some of these neuronal networks are so vulnerable is unclear - at least to me. B.t.w., the mouse does not have these centers (as it doesn't need to move the eyes), thus it cannot serve as model. In this respect, the chamaeleon would be a dream model. The new results show that a defect in the socalled *vertical supranuclear saccade palsy* is more reliable than the defect in the gaze palsy. This may sound like pea counting, but it may make a difference knowing what needs to be measured. The study also shows that some measures of eye movements correlate relatively well with other scales reflecting the severity of neurologic symptoms. The study reveals further what patients undertake – probably unconsciously – to compensate for their troubles with eye movements. These efforts can of course perturb the results of eye

exams depending on the patient. Of note: again, it would have been nice to see a multiparametric analysis to find out whether patients fall in distinct groups with respect to disturbances in eye movements and other neurologic parameters. In any case, it appears as useful to scrutinize eye movements (more) diligently - at least in patients where this is still possible. This may help to better diagnose the disease and to monitor for example effects of therapeutic drugs.

https://pubmed.ncbi.nlm.nih.gov/34387740

News about an old topic Targanil a.k.a. N-acetyl-L-leucine or NALL (see Pfrieger's Digest #4). The results of a clinical phase 2 study were published (Bremova-Ertl et al., 2021). The study bears some special points. First, patients received the L form (more precisely the L enantiomer; see last PD edition), which based on previous studies induces the effects. Second, the study is not placebo controled. Instead, it employs a before/after design plus a washout, a measurement after the treatment stopped. Third, the study uses a new kind of evaluation, where experts use videos to judge the severity of the neurological symptoms and their drug-induced changes blindly. "Blindly" because the experts did not know when the videos were recoded, before, after or after washout. The results show an improvement of symptoms during the daily intake of NALL and an impairment after washout. The question is still "how does it work". The authors mention – as others previously – energy metabolism as possible drug target.

Animal models

https://pubmed.ncbi.nlm.nih.gov/33810307/

The team of Merce Pallas from Barcelona (Spain) suggests a new therapeutic approach (Grinan-Ferre et al., 2021). The target protein is an enzyme, meaning a cellular machine, named *soluble epoxide hydrolase*, which mediates the degradation of specific signal components named *epoxyeicosatrienoic acids* (EETs). What's this all about? EETs are specific fatty acids that regulate different cellular processes such as mitochondrial function, autophagy, cell death and inflammatory responses. The use of these signals as therapeutic target is nothing new. Earlier studies showed positive effects in Alzheimer's disease, heart disease and cancer. The present study deals with NPC and shows that treatment of mice with an inhibitor of the enzyme prolongs the life-span of mice by one quarter and reduces some symptoms, namely behavioral changes. That's already something. The drug that was used is not well known. However, it is administered orally and seems to cross the blood-brain barrier. There's probably more to come.

https://pubmed.ncbi.nlm.nih.gov/33878344/

The fascinating study of Roney et al. (2021) presents a first explanation for a long-known phenomenon, the socalled axonal dystrophy in the brains of patients and of animal models. As reminder, an axon is a sort of cable that together with synapses and dendrites tranfers electrical activity from one neuron to the next. In humans, individual axons can be up to 1.8 meters or 6 feet long, the total length of the axonal wiring in the human brain measures – ATTENTION please – around onehundredeightythousand (!) kilometers corresponding to 111,000 miles. Dystrophy means basically a malnutrition or malformation. Axonal dystrophy, a kind of cable fault, in NPC was discovered many decades ago. It is characterized by an accumulation of vesicular structures at specific sites along axons. The reasons for this pathologic change and its consequences remained unknown for a long time, and admittedly, there was relatively little research into this topic. The work of Roney and colleagues now solves the riddle – at least partially. The team shows with an impressive array of experimental models and approaches that in NPC, the transport of lysosomes along axons is disturbed. Ok, one may ask, so what? Earlier work of the team had shown that lysosomes migrate into axons and synapses like a sort of "active trash bins", where they collect waste and transport this back to the cell body. There, the stuff is recycled or eliminated. If something goes wrong, there will be an accumulation of vesicles, specifically of autophagosomes, just because these vesicles cannot be removed by fusion with lysosomes. Interestingly, in NPC only the shuttle from the cell body to the cable ends is affected, whereas the back-transport seems functional. Why is this? Probably because specific proteins on the surface of the lysosomes that the motor needs for trailing are inactive. A reason could be the high cholesterol content of the lysosomal membrane. As always, such ground-breaking discoveries raise more questions, but a first step is done. And the work shows once more that nerve cells due to their special forms and functions have highly specific logistic demands and therefore suffer from highly specific defects in NPC that cannot occur in other cells.

https://pubmed.ncbi.nlm.nih.gov/34061502/

"**Fat in 3D**", this could be the headline of a new study of NPC1-deficient zebrafish (see last issue). Chinese colleagues used a relatively new and demanding method, named 3D MALDI-MSI, to visualize the distribution of lipids in healthy and diseased fish – in three dimensions. This results in beautiful pictures providing a total view on pathologic changes in different organs. It will be interesting to see that this innovative method will bring in the future.

https://pubmed.ncbi.nlm.nih.gov/34290407

Year after year – still too rarely – it happens: a publication in the NPC fields makes it into the premier league of scientific journals: This time NATURE. Jackpot! Congratulations to the team of Nan Yan at Texas Southwestern Medical Center in Dallas. They show something new with potential for a new therapeutic approach by linking two seemingly unconnected topics. First, NPC1 deficiency, and second the socalled *stimulator of interferon genes* (STING) signaling pathway. What's this about? Briefly, STING is one of those central switches in cells that control a whole range of cells - I spare you of details, it's a long and complicated story that I understand only partially. The team showed that the STING protein is directly linked to NPC1 and that the cellular level and activity of STING is increased, if NPC1 is absent. Overactivation of STING on the other hand may directly provoke death of neurons although it is not clear how. Possible via the release of different signals in response to inflammation. In any case, the team shows that NPC1-deficient mice are better off, if STING or components downstream of this signaling pathway are eliminated. Now, since STING plays a central role in several pathologic conditions including cancer, autoimmune and inflammatory diseases, several drugs were developed that inhibit this pathway. It will be interesting to see, whether treatment of NPC mice with these inhibitors slows down disease progression and prolongs their life span. Notably, the study suggests that STING activation and neurodegeneration are independent from lipid accumulation, an aspect that should be explored.

https://pubmed.ncbi.nlm.nih.gov/34364974

Self-promotion: a study from my lab asked what happens in the brain of NPC mice immediately after the administration of cyclodextrin (Barthelemy et al., 2021). So far, cyclodextrin-induced changes were investigated weeks or months after treatment. It was unknown what happens within hours and days after administration. Specifically, we aimed to test a hypothesis that came out of a previous study on cultured cells, where we showed that cyclodextrin enables neurons to spit out the superfluous cholesterol by a process known as lysosomal exocytosis. Does this happen in a living animal? To explore this, we used the retina as model. This part of the brain allows us to administer stuff like cyclodextrin directly by socalled intravitreal injections. Those are used for example to treat specific forms of macular degeneration in patients. Another big advantage is that we can inject control solution in the second eye, allowing us to study the control condition in the same animal. We found that cyclodextrin indeed induces the release of accumulated cholesterin from retinal nerve cells and that socalled glial cells clean up and digest the fat. Our data suggest that the removal of superfluous cholesterol requires a cooperation between

neurons and glial cells, probably because neurons are not able to manage the high fat load released by cyclodextrin. A reminder: neurons aren't fat cells!

https://pubmed.ncbi.nlm.nih.gov/34407999
There is progress at the gene therapy front. For this approach, the healthy version of the NPC1 gene is wrapped up in a socalled vector, very often *adeno-associated virus* or AAV for short. The vector is injected into the blood stream, from where it reaches different organs and channels the gene into the target cells including neurons – so much for the theory. First studies with promising results had been published (s. previous issues). The latest paper coming out of the Pavan/Venditti lab (Davidson et al., 2021) shows that a specific variant of the AAV vector named AAV-PHP.B is better than the classic version AAV9 due to distinct proteins on their surface, which mediate the viral entry into cells. The PHP.B variant prolonged the life span of NPC1-deficient mice much more than AAV9. However, one problem remained: variability. In some mice, AAV-PHP.B did wonders (longer life span, reduced neurologic symptoms) in others not. Why? This question was answered: apparently, the chosen NPC1 mouse model, which is based on the BALB/C mouse line, bears specific variants of the receptor that bind the viral surface proteins more or less well. Consequently, Davidson and colleagues showed that the effect of the virus-mediated therapy on the lifespan of a given mouse depended on the receptor variant that was present in the mouse. That's pure biology: First, things look terribly complicated, but once you dig deeper they appear (more) simple.

Cells
https://pubmed.ncbi.nlm.nih.gov/33921734/
A study from the Zanlungo lab (Argüello et al., 2021) shows that a plant hormone from soy beans, named *genistein*, reduces cholesterol accumulation in cell models of NPC. The authors demonstrate further that the drug activates a gene switch named TFEB, and thus controls the production of lysosomes. It is known that this switch also activates the release of lysosomes a.k.a. lysosomal exocytosis. What do we learn? Other studies showed that genistein activates several cellular processes, thus it is unclear, how specific the drug is. And it remains to be seen whether the results apply to "real cells" in the body. These caveats apply of course to many studies.

https://pubmed.ncbi.nlm.nih.gov/34019311/
A report from the lab of Eamon Dickson (Kutchukian et al., 2021) shows that defects in NPC1 protein can perturb a central signaling process that may serve as target for new therapeutic approaches. The story is complicated – it's biology! It is about phosphatidyl inositol, more precisely about phosphatidyl inositol 4 phosphate

PtdIns4P – admittedly that does not sound better. This lipid sits in the membrane of different organelles and controls the transport of - among other things - cholesterol. How's that? Well, this requires a sort of reverse transport, a favourite trick of nature. The principle: stuff A (cholesterol) present at high concentration at the start point (for example the lysosome) and low concentration at the target (for example the endoplasmic reticulum). Stuff B (PtdIns4P) present at high concentration at the target and low concentration at the start. Now, add several other components, membrane contact sites and a law of nature (stuff goes down concentration gradients, yes, thermodynamics...). The study shows that in NPC1-deficient cells, the metabolism of PtdIns4P is perturbed including concentration gradients and the enzymes that establish those. Whether a (therapeutic) repair of this mess would help, remains to be seen.

https://pubmed.ncbi.nlm.nih.gov/34023384/
https://pubmed.ncbi.nlm.nih.gov/33716137/

And we continue happily with fat. Two studies from the Storch team show that effects of NPC1 deficiency such as cholesterol accumulation and damaged autophagy can be diminished by adding phosphatidylglycerol (PG). How come? PG is precursor to *bis(monoacylglycero)phosphate* (BMP; a.k.a. *lysobisophosphatidic acid* or LBPA) – that doesn't make it easier. BMP/LBPA has a very special, trapezoid-like structure and lives probably exclusively in the late endosomes of cells. The Storch team had shown previously that BMP/LBPA helps to charge NPC2, the little sister (or brother) of big NPC1, with cholesterol. NPC2 passes cholesterol to NPC1, which in turn shuttles it out of the endosomal-lysosomal system. That's one idea. The latest works demonstrate again that increasing the concentration of BMP/LBPA – by providing the precursor – in NPC1-deficient cells reduces the accumulation of cholesterol! Bingo! So far, this was shown in cell cultures and one could say – so what! The new studies provide first albeit preliminary evidence that this works in living animals. Single injections of PG into the brain of NPC1-deficient mice caused a (small) decrease of cholesterol in cerebellar Purkinje cells. That ain't nothing!

https://pubmed.ncbi.nlm.nih.gov/34175669

A study from Spain deals with mitochondria further referred to as mitos (Torres et al., 2021). Since a long time, it is assumed that these cellular energy centers suffer in NPC disease. But the questions remain: to which extent in which cells. The new work suggests that one of the problems is uncontroled accumulation of cholesterol in mitos of NPC1-deficient cells. How this comes about isn't clear year, it may be a side effect: the lysosome runs full of cholesterol and in some cells some of the cholesterol flows to mitos. Mitos in turn suffer from the surplus cholesterol and do not function correctly, which then of course has catastrophic effects on the energy provision in cells. The study shows further that the enzyme *acid ceramidase* may help. This protein mediates the assembly and degradation of ceramide, a key component of sphingolipids. Defects in this enzyme cause morbus Farber. Increasing the amount of *acid ceramidase* in NPC fibroblasts seemed to normalize the function of mitos. These results suggest that the defect in mitos may be disconnected from the defects in the lysosomes and thus repaired independently – at least in fibroblasts. Whether this works in highly specialized cells of living animals, and whether this may serve as therapeutic approach, remains to be seen.

https://pubmed.ncbi.nlm.nih.gov/34183444

Interesting news about the NPC1 protein comes unexpectedly from a prominent corner of neuroscience (Guix et al., 2021). The Dotti team studied how and why nerve cells age. This is an important topic that's not easy to approach experimentally: you want to publish results bevor you get too old yourself. The Dotti group used cell cultures. That may seem a bit strange given that humans reach 80 plusminus x years, whereas cell cultures only a few weeks. But in the absence of alternatives... The study shows in neurons that the release of toxic stuff via socalled extracellular vesicles increases with age and that this increase has to do with an observed age-dependent decrease of NPC1 protein. Interestingly, the study shows that this decrease is controled by a well-known signaling pathway (Akt-mTor) and some less known pathways (socalled *microRNA repressors*). Whether NPC1 levels decline in neurons of aged animals remains to be explored.

https://pubmed.ncbi.nlm.nih.gov/34296265

The Pavan group investigated how cyclodextrin affects cells (Rodriguez-Gil et al., 2021). One could say: we know that! Well, on the one hand, yes, we know that cyclodextrin reverts cholesterol accumulation in NPC1-deficient cells. What we do not know is how exactly – well, there are different ideas. To address this question, the team performed a large transcriptome study using patient fibroblasts. This will tell us, which genes are up- and down-regulated after treatment with cyclodextrin in

cultured fibroblasts. Since the function of many genes is known, one can guess which processes or structures are affected. The study has three messages. First, cyclodextrin affected a limited number of genes and most were "among the usual suspects". This includes enzymes mediating cholesterol synthesis and components of lysosomes. Second, the results corroborate evidence published previously (s. Pfrieger's Digest #4) that the protein bearing the wonderful name *glycoprotein nonmetastatic melanoma protein B* or GNMPB can serve as reliable biomarker for NPC. Its concentration in the blood of NPC mice increased as the disease progressed and it decreased during treatment of mice with cyclodextrin or after virus-based gene therapy. The exact function of the protein is still unclear, it's probably a component of endosomes/lysosomes. The third message comes out from a multiparametric analysis (finally!): the genetic fingerprints of fibroblasts coming from different patients were remarkably distinct whereas the treatment with cyclodextrin introduced very small changes. Another hint that variability between patients may have to do with their genetic set-up.

Miscellaneous

https://pubmed.ncbi.nlm.nih.gov/33907281/

News from the marvellous kingdom of unicellular organism, here it's about a ciliated protozoan named *Tetrahymena thermophila*. This little "creature" is an important model organism for biological research, because it's easy to handle, because it bears all cellular components in one – no complications by livers, brains and such, and because it has some special features. One of them concerns sterol metabolism. Like many uni- and multicellular organisms ranging from yeast to humans Tetra needs sterols. Tetra solved the problem quite elegantly. If cholesterol is available from outside (don't ask me from where), Tetra will import it. The new study (Hernandez et al., 2021) shows that this import is mediated by a protein that is very similar to NPC1 – it still awaits identification though. By the way, if no cholesterol is available, Tetra presses a switch and manufactures its own sterol, named – guess how – Tetrahymenol.