By Frank W. Pfrieger
fw-pfrieger@gmx.de or frank.pfrieger@unistra.fr
Institute of Cellular and Integrative Neurosciences, CNRS, Strasbourg, France
(Translation: Kern AG supported by NPSuisse)

Dear Readers.
Many thanks for the positive feedback on the first issue of my "in-house tabloid" and a warm welcome to the second one. This admittedly unusual, but hopefully useful endeavour continues. My PubMed search again entailed the following terms: "**niemann pick type c OR niemann pick type C1 OR niemann pick type c2 OR npc1 OR npc2**". The second issue covers the period from April 1st to July 31st in 2020. For those in the know and those who aspire to be, here is the direct link:

((niemann pick type c OR niemann pick type C1 OR niemann pick type c2 OR npc1 OR npc2) AND (("2020/04/01"[Date - Publication] : "2020/07/31"[Date - Publication])))) NOT (("2020/01/01"[Date - Publication] : "2020/03/31"[Date - Publication])).

The search resulted in a total of **77** scientific publications in specialist journals. This means that an article appears every one or two days. Twelve of these are so-called reviews, i.e. overview articles summarising the current status of research with respect to a certain topic—from experts for experts. I will leave these out, as well as case studies in which clinicians describe individual patients. Notably, I only describe articles that I can read completely either via my institution access or as a PDF which the authors have sent me by request. My selection is subjective, and the following still applies: I have made efforts to make correct statements, but I cannot guarantee them. My assessments and interpretations are my personal opinion and make no claim to validity.

Patients
https://pubmed.ncbi.nlm.nih.gov/32073546/
A study at the NIH examined genetically diagnosed NPC patients aged between 6 months and 6 years. Of the 29 children examined, 25 showed enlarged liver or spleen and 26 neurological symptoms. Twenty-two of the children showed developmental delays which were so severe that they required therapeutic support, although only half of the patients received this. The authors underline the fact that the symptoms have to be examined with a series of tests entailing both motor as well as linguistic and cognitive performances. In addition, they emphasise that the connection between developmental delays and actual neurodegeneration is still unclear. So further studies on NPC patients with the early infantile form of sequence are required (see below).

https://pubmed.ncbi.nlm.nih.gov/32371106/
A large-scale international study with thousands (!) of Parkinson patients from Canada, France and Israel showed no connection between NPC1 gene variants and Morbus Parkinson. The reason for the examination was sporadic indications that on the one hand Parkinson patients bear NPC1 mutations and that on the other hand NPC1 patients manifest changes in the brain which are typical for Parkinson.

https://pubmed.ncbi.nlm.nih.gov/32324281/
https://pubmed.ncbi.nlm.nih.gov/32334605/
Marc Patterson and colleagues published two articles co-financed by Actelion in which the effects of Miglustat were demonstrated on the basis of the patient register kept from 2009 - 2017. The results of the first study indicate that Miglustat/Zavesca extends the lifespan from occurrence of the neurological symptoms - to be precise they indicate that the drug reduces the probability of death. However, the effect was only statistically significant in patients with the late infantile form - this group represents the majority of the patients listed in the register. Merely a tendency could be seen in the other groups. If on the other hand the time from diagnosis (and thus probably also from Miglustat treatment) was examined, things looked somewhat better. You just have to decide at what you look.

https://pubmed.ncbi.nlm.nih.gov/32349180/
In this study, the group of Elisabeth Betty-Kravis reports on the experience with total anaesthesia which was used during cyclodextrin administration in pediatric patients. For this, data from 394 treatments on 19 patients taking place between 2015 and 2019 according to the same protocol were evaluated, a substantial number! The vast majority of the treatments (349) involved inhalation masks with so-called supraglottic airway devices, i.e. laryngeal masks, being used for the rest. To cut a long story short, five cases resulted in severe undesired effects, amongst them choking, and 19 cases showed less severe side effects, amongst them vomiting and hypotension. All told, the authors draw a positive balance, total anaesthesia by inhalation appears to be safe and efficient for pediatric patients.

https://pubmed.ncbi.nlm.nih.gov/32592146/
Mandia and colleagues examined whether new potential NPC suspicion cases can be recognised and then purposefully verified by genetic tests with the new biomarkers for NPC, i.e. Oxysterol (C-triol), Ketosterol and LSM-509. For this, adult patients presenting neurological or psychiatric symptoms since the age of 12 were looked for all over France. Of the total of 251 examined patients, six showed increased values of one or more marker(s), two of this group had NPC. The study revealed false-positive rates for the various markers (LSM-509: 1.2%; Keto: 8.1%; C-triol: 5.7%) and showed

that the diagnostic tests are well suited to recognise suspicion cases relatively easily and reliably.

Animal models

https://pubmed.ncbi.nlm.nih.gov/31707730/

The group of Charles Vite examined in cats how cyclodextrin spreads in the body following injection into the cerebrospinal fluid. The injections were done either into the so-called Cisterna Magna, i.e. between the first cervical vertebra and the occipital bone, or by lumbar puncture at the level of the lumbar vertebra. To render the cyclodextrin "visible", it was marked radioactively and then "photographed" in sections of the various tissues with a pertinent scanner. The results are interesting: firstly, the cyclodextrin concentration reaches the highest value after one hour in the brain regions where it arrives following injections into the Cisterna Magna. It mainly reaches regions on the surface of the brain, but hardly parts located more deeply such as the thalamus. If the cyclodextrin is administered via lumbar puncture, even less reaches the brain - evidently, because the injection point is further away. On the other hand, cyclodextrin is found in the blood within minutes and in the urinary bladder after an hour, so a large part is excreted quickly. Mind you: the examinations were done on healthy cats. With NPC cats, that may be different, the cyclodextrin may stay in the cells for longer periods. The observation that a surprisingly large amount of cyclodextrin reaches the nasal cavities is very interesting. Why? Well, there is a relatively open connection between the air space in the nasal cavity and the olfactory cells. This suggests that administration of drugs via the nasal cavity into the brain might be practicable.

https://pubmed.ncbi.nlm.nih.gov/32291331/

A study from Maastricht in the Netherlands asked whether a diet enriched with plant stanols, a specific class of sterols, has positive effects in NPC1-deficient mice. That actually appears to be the case, at least as far as the so-called "peripheral", i.e. non-neurological symptoms are concerned. Animals on a specific diet firstly via their mother's milk and then directly via food pellets from the 12th day of life showed reduced liver weight and lower cholesterol concentrations in the blood and in the liver compared to control animals. In addition, inflammation values were improved. The reasons for these changes are not quite clear. The plant stanols may reduce the intake of cholesterol or they may have a direct anti-inflammatory effect. All of this was found in mice, but it remains unclear whether and to what extent the results can be translated to patients.

https://pubmed.ncbi.nlm.nih.gov/32342600/

This article is being mentioned out of self-interest. Colleagues from Japan describe ultrastructural changes in nerve cells of NPC patients and NPC1-deficient mice, i.e.

changes at the nanometre range which can only be detected by electron microscopy. Cholesterol and other fatty substances accumulate in lamellar structures which look very similar in man and mice. Unfortunately, it must be stated that electron microscopic examinations of autopsy material are a bit absurd. It's almost like wrinkles on your face; the better the magnifying glass you use, the more you discover. With an electron microscope (THE magnifying glass per se) all you detect in autopsy material is cell debris, because much too much time passes between death, removal of the tissue and its processing for electron microscopy.

https://pubmed.ncbi.nlm.nih.gov/32371566/
Basically, we always talk about fatty substances accumulating in cells in NPC. That's what you think! A new study by the Cologna group shows that the content of certain so-called phospholipids in the cerebellum of NPC mice drops drastically. The biology of these phospholipids is extraordinarily complex - too complex for this author. The finding that something is wrong with these substances is of great importance, as certain forms of the so-called phosphatidylinositols are extremely important for the control of fundamental cellular processes. Possibly, phospholipid-dependent signal paths are then also a target for new therapeutic approaches.

https://pubmed.ncbi.nlm.nih.gov/32417449/
A study by Rallapalli and colleagues from New York examines the changes in the brains of NPC1-deficient mice with the help of magnetic resonance imaging. The authors show that the volume of various brain regions in the NPC1-deficient mice increases from the third to the sixth week, similar to their healthy and heterozygotic comrades. However, on a much lower level. Between the sixth and the ninth week, it then shrinks, unlike the control animals. The interesting thing is that not only the cerebellum gets smaller, but many other regions of the brain as well. It would be nice to see the volume changes in individuals to learn about inter-individual variability.

https://pubmed.ncbi.nlm.nih.gov/32611604/
It is true: NPC mainly affects children, but we know ridiculously little about how the disease affects brain development. The authors have addressed precisely this question with the help of the so-called nmf164 mice. These mice have a point mutation, which leads to the NPC1 being broken down at an early stage; they show a slower sequence of the disease than the aforementioned Balb/c line (precisely: BALB/cNctr-Npc1^{m1N}/J), which lack the NPC1 protein altogether. On the other hand, this choice is not really all that obvious: if you want to examine changes in the development, should you not better look at mice with a faster progression? Who knows! The authors find that the "broken" NPC1 disturbs the development of the so-called microglia cells. "So what?", the layman may ask. These cells have been written off as the brain's dustmen for years, as if they were only keeping the brain clean. But,

as so often in biology, this idea is way too simple. In the meantime, it has become clear that these cells have much more cunning tasks: they can actively bring about the death of nerve cells, eliminate superfluous synapses during puberty and decisively control inflammation reactions in the brain. So the dustmen are also doing at least landscape gardening tasks on top. The study shows that not only the development of the microglia is disturbed in NPC 1 mice, but also their "feeding behaviour". The latter for its part leads to errors in the synaptic wiring of Purkinje cells. It is not yet clear whether and how these changes affect the further development and in the long run contribute to the neurological symptoms.

https://pubmed.ncbi.nlm.nih.gov/32731618/
Microglia and other brain cells are featured in a new study from Denny Porter's laboratory, a so-called "single-cell transcriptome" analysis. What's that? Here, the authors examined how lack of NPC1 affects individual cells of the mouse cerebellum. This is the first study of this kind published, others will doubtless follow, as single cell transcriptomics studies are shooting up all over the place. They are extraordinarily important, as they reveal how all the highly specialised cells in the brain react to whatever you want (development, pathology, medications etc.). The results show - somewhat surprisingly - that possibly not all that much is happening within the time period studied. The authors find relatively few genes, the expression of which changes in NPC1-deficient animals compared with the "normal" ones. A distinct reaction is shown - back to the topic mentioned above - by microglial cells. In these cells, some changes are seen in mice as young as three weeks old. The results together with the study already mentioned put microglia on the radar: these cells have to be examined more closely. It must be stated that such studies still face several methodical hurdles, for example that too few cells of the same type were analysed. However, these hurdles will without doubt be overcome in the future.

Cells
https://pubmed.ncbi.nlm.nih.gov/32144825/
Each of us knows some kind of "wild types" - or has even been together with one. Elina Ikonen's group in Finland asked whether the wild-type of NPC1 is really wild-type. In fact, there is a version of the wild-type, i.e. "normal" gene of NCP1 that is used in many laboratories, the sequence of which however deviates from the so-called reference version. It ought to be stated here that the "normal" version in genes/proteins - amongst them also NPC1 - probably does not exist, there are innumerable variants that have accumulated during evolution by mutations in the genotype; some of them cause problems = illness, whereas others improve the function. The study on cell lines showed that even the often-used wild-type version of NPC1 does not eliminate the cholesterol from the endosomal system as efficiently as the reference version. Computer simulations indicate that the assumed wild-type

manifests structural changes in the NPC1 protein. As a result it gets caught in the endoplasmatic reticulum and transports cholesterol less quickly. The study also shows that one can do useful things with cell lines, it depends on the question.

https://pubmed.ncbi.nlm.nih.gov/32248828/
The study by Musalkova et al. (2020) from Prague must be mentioned in this context. The authors examined whether the severity of the illness correlates with specific measures in skin fibroblasts from 26 NPC patients with different gene variants. This question is a core problem with NPC and the current opinion states that there is hardly a connection, although this has rarely been examined systematically. The authors at least find a loose connection between the amount of NPC1 protein available in the cells and the NPC type (early, late, juvenile and adult). Other measurements are also correlated more or less loosely. Two remarks: the way how the amount of protein was measured is standard, but it is not very precise. In addition, what was stated above also holds true here: the scientific question is key! Are fibroblasts the best model to answer the question? Probably not, as neurological or psychiatric symptoms originate in cells in the brain, not in those of the skin. New approaches and more work are needed!

https://pubmed.ncbi.nlm.nih.gov/32385114/
Back to the subject of "surprise": a study from the Spiegel group shows a new connection between the so-called sphingolipids and cholesterol. The former also belong to the group of lipids with "complicated biology". The accumulation of sphingolipids in NPC is nothing new. However, the study shows that pharmacological activation of the so-called sphingosine kinase, which helps to break down sphingolipids, reverses the accumulation of cholesterol in fibroblasts with defective or even missing NPC1. Evidently, it will be important to see whether such effects can also be seen in animal models.

Miscellaneous
https://pubmed.ncbi.nlm.nih.gov/32396202/
News from the animal world: Chinese researchers report that the Chinese tussar moth (*Antheraea pernyi*) possesses a protein similar to NPC2 that controls its larval development together with steroid-like hormones.

https://pubmed.ncbi.nlm.nih.gov/32544384/
There is a whole series of new studies on the structure of the NPC1 protein. They address the fundamental question how this protein together with its smaller sibling, NPC2, heaves cholesterol across the membrane of the endosomal-lysosomal system. There is evidence that cholesterol enters a tunnel within the NPC1 protein, although it is unclear how and where it comes back out again. The study by Nieng Yan in

Princeton is mentioned representatively for all the others. Her latest paper indicates that the passage through the tunnel depends on the pH, i.e. how acid the environment is. It is known that the lysosome is very acid to facilitate breakdown of material (pH 5.5 and below). The pH dependence appears as a smart evolutionary trick: it prevents the protein from being active and from pushing cholesterol all over the place before it reaches its final workplace in the endosome-lysosome.

https://pubmed.ncbi.nlm.nih.gov/32556727/

Last but not least, some information for seafood lovers: a study from Thailand indicates that NPC2 plays a role in the maturation of shrimp sperm, possibly by reducing its content of cholesterol.