Pfrieger's Digest

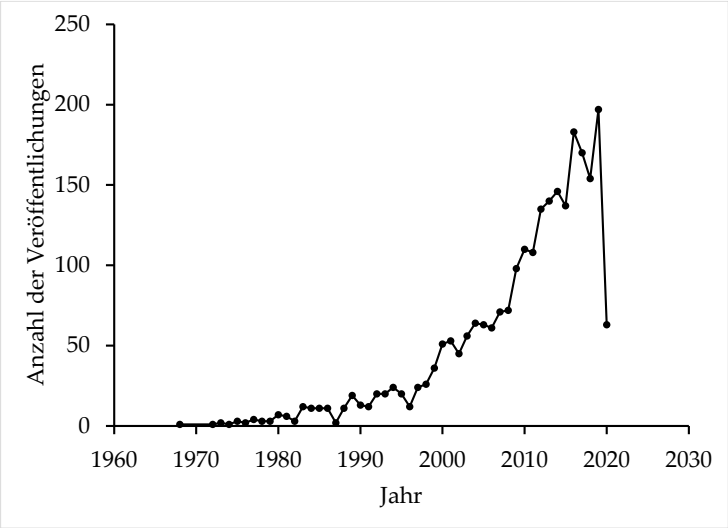
Issue 01 / 2020

Dear Readers,

What you are looking at now is the very first issue, fresh from the press, of my new periodical appearing three or four times a year. With this kind of house dispatch, I would like to inform you about the latest results of NPC research - I hope regularly. In the medium term, it will take over from my annual conference reports and make more topical and also broader reporting possible. For background information, I make reference to the conference reports (feedback please to fw-pfrieger@gmx.de).

As the title - please note *digest* and not *digestion* - suggests, this is a personal selection, so there is no claim to completeness. Nevertheless, this *digest* is naturally based on "genuine" publications which are listed in the Pubmed database, which is accessible to everybody. Most of the papers have been *peer-reviewed*. This means that the results and also their presentation and interpretation have been reviewed anonymously by at least two, often even three specialist analysts, who then possibly demand corrections or extensions. As with the conference reports, this also applies here: I have endeavoured to have correct statements, but I cannot guarantee them. May assessments and interpretations are my personal opinion and make no kind of claim to validity.

My Pubmed search entails the following terms: "**niemann pick type c OR niemann pick type C1 OR niemann pick type c2 OR npc1 OR npc2**". I chose the period from 1 January until 31 March 2020 for this issue. The search resulted in a total of **63** scientific publications in various specialist periodicals. Patient-relevant results are stated first, then studies on animal and cell models and also "Miscellaneous". I only mention studies which I can also read via my access in the institution, some periodicals are therefore not taken into account. As is customary, the studies are quoted with the first author's surname, the remainder "et al." for more than two authors and the year.



The illustration shows how the number of scientific articles on the subject has developed in the course of the year. The almost exponential sequence of such graphs is also recognised by inimical laymen - thanks to Covid-19. The figure for 2020 naturally only reflects the number from January to the start of April.

[Left: Number of publications Bottom: Year]

Patients

https://www.ncbi.nlm.nih.gov/pubmed/28865947

Studies by Spanish colleagues (2 x Lopez-de-Frutos et al., 2020) describe new variants of the NPC1 protein, amongst them the case of a 26-year-old NPC patient suffering from neurological and psychiatric symptoms and manifesting a known variant (p.Ile1061Thr) and one unknown up to now, p.Val856Ala.

https://www.ncbi.nlm.nih.gov/pubmed/31639880

With the help of magnetic resonance tomography (MRT), a group from Germany (Gburek-Augustat et al., 2020) was able to show that NPC patients with varying starts of the illness, i.e. early/late infantile, juvenile etc., also manifest various changes in certain regions of the brain. This is a further indication that MRT may possibly serve to monitor the sequence of the illness in the brain non-invasively - i.e. without an intervention - and to verify possible effects of treatments.

https://www.ncbi.nlm.nih.gov/pubmed/32033912

An interesting study (Sidhu et al., 2020) is concerned with the age-old subject of biomarkers, i.e. substances or mixtures which are eagerly longed for and serve diagnosis and monitoring of the condition of NPC patients. Some time ago, a new marker, the so-called LysoSM-509, was presented, it appeared to be a descendant of the famous sphingomyelin, i.e. of a component of the cell membrane closely connected with cholesterol. However, the structure of this new molecule remained unknown. This prevented artificial production of the substance and the precise determination of his concentration in patients' blood. To put it in a nutshell: last year, the structure was clarified and came as a real bombshell: it is a question of a "fat" unknown up to now by the name of N-palmitoyl-O-phosphocholineserine or in short PPCS. The current study confirms that the PPCS concentration is actually very much higher in the blood of NPC and possibly also ASMD patients, but not patients with other disorders or healthy volunteers. Therefore, PPCS is very specific for NPC. The concentration is blood is however not greatly influenced by cyclo-treatment of the brain (lumbar puncture). Where and how this molecule is precisely produced and what functions it has is not yet clear.

https://www.ncbi.nlm.nih.gov/pubmed/32138288

A study by Dardis et al. (2020) summarises the molecular genetic examinations of all the 105 NPC patients known in Italy. The authors emphasise that the number of diagnosed adult patients is increasing, that the normally frequent mutation I1062T is seen relatively rarely and that a large number of differing variants can be found instead. At the same time, new variants are being discovered. It is seen once again that other genetic factors also clearly determine the symptoms.

https://www.ncbi.nlm.nih.gov/pubmed/32178982

A study from the United Kingdom draws a balance (Cooper et al., 2020) as far as oxysterol-based diagnosis is concerned. After five years, the authors come to the conclusion that the test reliably recognises NPC patients, but that also wrongly negative (NPC not recognised)

and falsely positive results are possible, as patients with other diseases such as NP A/B and Morbus Wolman likewise show increased figures.

https://www.ncbi.nlm.nih.gov/pubmed/32209649
Mark Walterfang's group (Walterfang et al., 2020) shows an increased neuro-inflammation in the white substance of the brain in a new PET scan study on adult NPC patients. To remind you, a part of the neuro-inflammation, as it were the inflammation reaction in the brain, is activation of so-called microglia cells, which can be measured via a certain substance which is administered to the volunteers. Compared with control volunteers of the same age, cells of the white matter were more strongly activated than those of the grey matter in patients. The white matter mainly entails nerve cables. At the same time, a reduction of the volume of these brain areas was observed, although it did not appear dependent on the severity of the illness. The paper shows once again that imaging methods can possibly be used for effectivity tests - albeit only together with other measurements (biomarkers!).

https://www.ncbi.nlm.nih.gov/pubmed/32222928
A study which is very pleasing for us comes from Munich. It shows that NPC patients actually manifest pathological changes to the retina (Havla et al., 2020). Our examinations on the mouse model had already forecast this as far back as 2009. The present study confirms this: with the help of so-called optical coherence tomography (abbreviated also OCT) - it sounds worse than it is - it shows that certain layers of the retina in NPC patients are thinner than in control volunteers. This non-invasive examination of the retina is therefore possibly - just like the aforementioned PET and MRT - a practicable way in order to monitor the progress of the illness and to examine possible therapeutic effects.

https://www.ncbi.nlm.nih.gov/pubmed/32234823
The same groups from Munich also published a study on 20 clinically inconspicuous heterozygous carriers of NPC1 mutations (Bremova-Ertl et al., 2020). With this, possible early indications of neuro-degeneration were to be discovered. In some of the volunteers, this test actually showed conspicuous changes to eye movements, in cognitive tests as well and also in the metabolic activity in certain regions of the brain, above all the cerebellum.

Animal models
https://www.ncbi.nlm.nih.gov/pubmed/31312996
Stephen Turley's group reports on a slow accumulation of cholesterol in the small intestine of NPC1-deficient mice (Balb/c Model) (Lopez et al., 2020). This change is weakened by treatment with ezetimibe, which inhibits the cousin of NPC1, the NPC1L1 protein, and cyclodextrin. The effects of the NPC1 malfunction on the small intestine have not been examined very extensively up to now.

https://www.ncbi.nlm.nih.gov/pubmed/31668555
A study from the laboratory of Denny Porter is concerned with the quite important question of why NPC patients show such great differences in the symptoms and the sequence of the

illness (Cougnoux et al., 2020). Whereas other genetic factors, i.e. individual differences in the genetic make-up, are high on the list (see above), there are naturally also environmentally induced candidates. These include pre-natal infections or similar situations which activate pregnant women's immune systems. To verify this, NPC1-deficient pregnant mice (nih, Balb/c) were confronted with an agent which artificially triggers a strong immune reaction, i.e. practically imitates a viral infection. This intervention deteriorates the course of the illness in female next-generation mice slightly, but nevertheless measurably. It naturally remains unclear up to now whether this also applies to humans - the question of transferability of results between animals and humans. Environmentally induced influences on the sequence of the illness are however still in the running.

https://www.ncbi.nlm.nih.gov/pubmed/31862414
A study on mice with the so-called nmf164 mutation, which shows a slower sequence of the illness than the NPC1-deficient Balb/c mice (nih allel), confirms a lower function of mitochondria in the liver (Erickson et al., 2020). Mitochondria guarantee the supply of energy to the cells and thus have a key position, for example for nerve cells which are hungry for energy. The changes affect younger mice in particular. Strangely enough, the malfunction reduces in older age. So the liver appears to be in a position to counteract it.

https://www.ncbi.nlm.nih.gov/pubmed/31937940
News from the genetic therapy area: a group at the American Harvard University reports on a new approach to repairing genetic defects in general. The approach is based on artificial helpers, the so-called base editors. These enzymes, which are based on CRISPR-CAS, can correct the genetic make-up directly, that is to say, for example, replace letters (the bases) (Levy et al., 2020), a kind of Tipp-Ex for DNA. Naturally, these helpers have to be channelled into the cells, which really ought to function with the famous AAV virus. But unfortunately, the DNA with the blueprint for the enzymes does not fit into the virus, it is too long. The group was now able to show that the blueprint can be divided onto two viruses. Cells which have been infected with both viruses can then build the enzymes on the basis of the blueprint halves which have been joined. Unfortunately, the paper is not yet accessible and is under embargo until June 2020. That happens above all when it is assumedly hot stuff.

https://www.ncbi.nlm.nih.gov/pubmed/31996359
A very interesting article from Bill Pavan's laboratory presents a new mouse model for NPC (Rodriguez-Gil et al., 2020). Above all, it brings important new indications that the sequence of the illness is influenced by genetic factors (see above). The results confirm that the life expectation in mice changes if the same NPC1 mutation is hybridised into various strains of mice. That is about the same as if an aggressive species of dogs is hybridised with any other species and we establish that the next generation is as meek as a lamb. Here too, genetic factors play a role. Naturally, the million dollar question is which factors influence the NPC symptoms. Here, the study goes an important step further. It shows firstly that there are a number of places in the genetic make-up which change the lifespan of the NPC and secondly that these factors are additive and that thirdly they can be found on mouse

chromosomes 1, 7 and 17. Now, chromosomes are very long and contain thousands of genes and regulatory sections. Identification of these factors and the important proof that and how they change the symptoms of NPC - at least in the mouse model - are now within reach. The necessary experiments are very time-consuming and demand hosts of animals, so it will take some time yet.

Cells

https://www.ncbi.nlm.nih.gov/pubmed/31489965

A study from Japan examined on a cell line whether and how sphingomyelin influences the accumulation of cholesterol (Wanikawa et al., 2020). Both lipids accumulate in the cells, but the extent to which the accumulation of one induces that of the other is unclear. A real chicken and egg problem. Unfortunately, such examinations on cell lines are to be treated with caution as their transferability to "normal" cells in a living body is very dubious.

https://www.ncbi.nlm.nih.gov/pubmed/31509197

In this paper, Bill Balch's group shows that a chemical compound with the name JG98 possibly reverses the accumulation of cholesterol in fibroblasts of NPC patients (Wang et al., 2020). However, this only affects mutations which lead to errors in the folding of the NPC1 protein and thus to its degradation. JG98 inhibits certain components of the so-called heat-shock protein 70 within the cell and is currently also being tested as a medicine for cancer therapy. Tests are necessary as to whether the substance also works in an animal model or even with patients.

https://www.ncbi.nlm.nih.gov/pubmed/31988149

A study from Harvard is causing stomach pains for the author of these lines (Feltes et al., 2020). It is a question of the fundamental question of how cyclodextrin reverses the pathological accumulation of cholesterol in cells. There are - as so often - contradictory results. The group refers back to previous studies, amongst them one by us, and follows up the question of whether cyclodextrin triggers the direct release of cholesterol from the cell. As a model, the group uses a cell line - as many others before - and finds that cholesterol is actually released, but by a mechanism other than the one which previous studies including ours showed on freshly isolated nerve cells. Now in principle, that doesn't bother anyone, but it does raise fundamental questions with which all of biomedical science is confronted: does what we find out by examinations on cell cultures also apply in living animals and does what we observe in animals also apply to humans? How much confusion is caused by the fact that research is done on unsuited models alone?

https://www.ncbi.nlm.nih.gov/pubmed/32072649

The study by a Danish group (Hede et al., 2020) tries a new approach to correcting the genetic defect for Niemann-Pick type C2. To remind you: NPC2 is the "soluble" and non-membranous sparring partner of NPC1 and forwards cholesterol to the latter. The protein is small, but it does not pass the blood-brain barrier. Therefore, a relatively simple substitution therapy cannot function, the same problem as with Niemann-Pick Type A or A/B. The group's idea is now to get the cells which form the blood-brain barrier to produce NPC2

themselves and then to release it into the brain. The study shows in cell cultures that this possibly might work, but unfortunately the amount of protein is still much too low.

Miscellaneous

https://www.ncbi.nlm.nih.gov/pubmed/31711697

And here is a report from agriculture, for pig breeders and other interested parties on the basis of a study from Colombia (Valencia et al., 2020). It was proven that the amount of NPC2 determines the freezing capacity of pig's sperm; the more, the better. They do not yet know why this is. And also not whether it also applies to other animals or humans.

https://www.ncbi.nlm.nih.gov/pubmed/31940478

More from the animal kingdom (Takadate et al., 2020): bats, which are known to be transmitters of Ebola and Marburg viruses, manifest differences in the NPC1 protein and these differences determine whether the bat can be infected by one or another virus.

"niemann-pick type A" OR "niemann pick type B" OR "niemann pick type A/B" OR (smpd1 AND (disease OR disorder OR deficiency)) OR (acid sphingomyelinase AND (disorder OR disease OR deficiency))