Pfrieger's Digest for Niemann-Pick Type A-C
Summaries of new research advances related to Niemann-Pick diseases based on selected peer-reviewed publications in scientific journals.

By Frank W. Pfrieger, PhD
Institute of Cellular and Integrative Neurosciences
Centre National de la Recherche Scientifique / University of Strasbourg
Strasbourg, France.

Dear Readers.
Welcome to this new issue of Pfrieger's Digest, which covers the period from May 1st 2022 to September 30th 2022, and which includes now publications related to **Niemann-Pick type A-B** called acid sphingomyelinase deficiency. The following abbreviations will be used: ASM for the enzyme acid sphingomyelinase, ASMD for the disease acid sphingomyleinase deficiency, and NPCD for Niemann-Pick Type C disease. The links for the PubMed queries are:
- for NPCD:

((niemann-pick c OR niemann-pick type C OR niemann-pick type C1 OR niemann-pick type c2 OR npc1 OR npc2) AND (("2022/05/01"[Date - Publication] : "2022/09/30"[Date - Publication]))) NOT (("2020/01/01"[Date - Publication] : "2022/4/30"[Date - Publication]))

- for ASMD:

((niemann-pick AND ("type a" OR "type B" OR "type A/B") OR smpd1 OR asmase OR acid sphingomyelinase) AND (("2022/05/01"[Date - Publication] : "2022/09/30"[Date - Publication]))) NOT (("2020/01/01"[Date - Publication] : "2022/4/30"[Date - Publication]))

During the period, **63** (NPCD) and **39** (ASMD) articles were published in scientific journals including **7** (NPCD) and **7** (ASMD) reviews. Seven articles belong to both areas. Note that queries in PubMed cover a large fraction of the scientific literature, but not everything. As you may know, there are other bibliographic databases such as Web of Science, Scopus and Google Scholar. Respective query results overlap largely, but not completely: publications listed in one database can be absent from another. Still, PubMed delivers most of the relevant literature, it has powerful search functions, and it is freely accessible.

The following applies as stated in previous issues: 1) My selection is subjective. 2) I do not comment on review articles or case studies. 3) I only describe articles to which I have access or I receive upon request to authors. 4) I try to ensure

correctness of statements, but I cannot guarantee this. 5) My judgements and interpretations expressed are subjective and reflect my personal opinion, they do not claim any validity and they may be erroneous. 6) I apologize for errors (grammar, orthography etc.) and any wrong, quirky or otherwise weird expressions. As for previous issues, this is my translation of my original German version.

Please feel free to distribute and forward this issue, and to send feedback to: fw-pfrieger@gmx.de or frank.pfrieger@unistra.fr. Suggestions for changes/improvements are highly welcome, notably for a better translator than me - das wär' wunderbar!
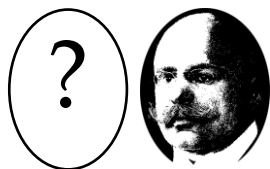
Patients (NPCD)

https://pubmed.ncbi.nlm.nih.gov/35892469/

A Brasilian study (Kubaski et al., 2022 Int J Neonatl Screen) reports results of a two-step protocol to diagnose NPCD that is based on the biomarker lysosphingomyelin-509. The correct name of this newly discovered lipid is N-palmitoyl-O-phosphocholineserine or PPCS (see also Pfrieger's Digest 1). The study used 450 dried blood spots from Brasil, Bolivia, Kolumbia and Ecuador from patients with suspicion of NPC. Out of 33 samples with elevated PPCS values, 25 patients were diagnosed with NPCD based on genetic tests. Exams for the remaining samples are still in progress. The study indicates that PPCS allows to detect NPCD patients reliably even from dried blood spots, and under logistically difficult conditions such as large geographic distances in South America.

https://pubmed.ncbi.nlm.nih.gov/35833379/

Same topic: a group in the UK reports results of a large biomarker study with 75 children and teenagers, who entered hospitals with a suspicion of neurometabolic disorders (Papandreou et al., 2022 Dev Med Child Neurol). The study shows that a combination of blood biomarkers including the above mentioned PPCS and others allows for a more reliable diagnosis of NPCD than each marker alone. The keyword here is multivariate analysis. This approach looks at all markers at the same time using special statistical methods (for connoisseurs: principal component analysis). This type of analyses gains importance in biomedicine, as the number of parameters increases and single measures cannot reveal "the whole picture" (see also Pfrieger's Digest 5, and the next but one article).

https://pubmed.ncbi.nlm.nih.gov/35945383/

A study from Australia deals with the challenge to record movement disorders in NPCD patients (El-Masri et al., 2022 Neurol Sci). A group with Mark Walterfang put its hand into the toolbox for morbus Parkinson. They tested a machine named

Personal KinetiGraph (PKG) in twelve NPCD patients at 18 or more years of age. PKG is a wrist watch-like tool, which is distributed by an Australian company named Global Kinetics. PKG records over a defined period, 6-7 days, all movements of the patient. The data are transmitted and analysed by company-owned algorithms. The analysis delivers a wealth of parameters (bradykinesia, tremor, immobility, and sleep behavior) describing the patient's disease condition. The study shows the utility of the machine to gauge movement disturbances in NPCD patients. Evidently, there is a need for more studies with more patients, and possibly a need to adapt the algorithms due to different symptoms and disease progression in NPCD compared to Parkinson.

https://pubmed.ncbi.nlm.nih.gov/35961091/

Back to the diagnosis challenge. It's getting complicated raising the immediate question how one can explain something that one hasn't understood. One solution: make it short! Spanish and British authors introduce a refined approach to identify NPCD patients (Moreno-Barea et al., 2022 Comput Biol Med). The basis of the study were socalled "metabolomics" data of 13 NPCD patients lacking medical treatment and 47 heterozygous carriers. Their dataset, concentrations of different metabolites in urine samples, was mashed with different artificial intelligence tools. One of those is named data augmentation. Here, a dataset that is limited by numbers (rare disease = few patients!) is artificially "augmented" using mathematical models. These data were then fed into a machine-learning approach using neuronal nets plus other algorithms - to make it short. Essentially, the point here is to uncover complex patterns in the metabolite concentrations that distinguish patients from all others. What came out of this? The approach seems to work. It uncovered new urine biomarkers that may facilitate diagnosis and indicate disease progression. As stated above, this kind of approaches will gain importance in the future last not least for rare diseases with few patients and a large variation of individual parameters.

https://pubmed.ncbi.nlm.nih.gov/36064725/

A study from NIH summarizing data from 120 NPCD patients provides a comprehensive view on swallow dysfunction (Solomon et al., 2022 Orphanet J Rare Dis). An earlier publication from 2020 (see issue 3) revealed effects of Miglustat/Zavesca. Swallowing was recorded by videofluoroscopy. From the movies and other exams a wealth of parameters was extracted and summarized in severity scales. The results show that the time to swallowing decline and its further development varies from patient to patient. Factors that influence the swallowing dysfunction are time - the longer the worse, and seizures - the more the worse. Moreover, around 12% of the recordings showed silent aspiration. This symptom is
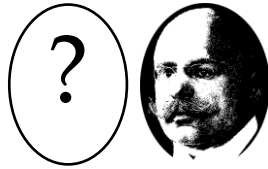
often overlooked in basic swallow exams, although it requires immediate action. The authors recommend regular exams of swallowing function with videofluoroscopy depending on the clinical picture, in addition education of patients, family members and caregivers about swallowing function, precautions and protective measures including alternative feeding methods.

Patients (NPCD + ASMD)
https://pubmed.ncbi.nlm.nih.gov/36056366/
The article of Diaz and colleagues (Diaz et al., 2022 Orphanet J Rare Dis) summarizes the results of a poll asking about health insurance literacy among ASMD und NPCD patients and their caregivers in the USA. This included difficulties to obtain health insurance and access to healthcare services. Not surprisingly, it shows that the community is more familiar with these matters than the general public. On the other hand, there are big knowledge gaps and difficulties with calculations of health insurance and with reception of medical treatments and assistance. The paper documents that delays and declines of medical help worsen the condition of patients and the quality of life of caregivers, the keyword here is "burnout". There is much to be learnt to navigate healthcare systems, which are complex worldwide.

Animal models (NPCD)
https://pubmed.ncbi.nlm.nih.gov/35507892/
A study from the Chang lab at Dartmouth (Rogers et al., 2022 Proc Natl Acad Sci USA) deals with factors that impact disease progression and severity and that may serve as new targets for therapies. Prime candidates are those proteins that are related to cholesterol. A main actor in this cholesterol theatre is the acyl-coenzym A:cholesterol acyltransferase 1 (ACAT1 or SOAT1). This enzyme converts cholesterol to a special form, a socalled cholesterolester, that can be stored by cells and transported in between them through lipoproteins. For this conversion, a fatty acid is married with cholesterol via the OH group. This modification renders cholesterol more water insoluble: it cannot stay in membranes and has to move to lipid droplets. This modification is long known, but how much of this happens in which cell of the body is unclear, notably in brain cells, where everything is different anyway. The new study shows in NPC1-deficient mice that additional elimination of ACAT1 prolongs their lifespan and reduces damage to the liver and brain. This suggests that esterification of cholesterol aggravates disease progression, although it is unclear why and how. Much remains to be learnt even for a well-studied process.

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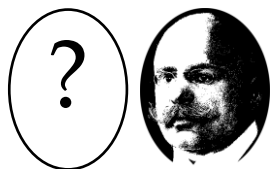
A new study from the Porter lab at the NIH also aims at factors that determine how NPC1 deficiency affects cells (Cawley et al., 2022 Int J Mol Sci). Their interest focused on the lysosome, and more precisely on their interior sugar-icing. The icing protects lysosomes from the acid sauce and the aggressive enzymes that degrade proteins and lipids, and thereby risk to digest the lysosome from inside. The sugar icing consists of distinct molecules that are bound to lysosomal proteins thus creating a thick protective layer – here, the comparison to icing breaks down, as the latter doesn't really protect those pastries from being eaten. The group found earlier disease onset and shorter lifespan in NPC1-deficient mice, if they lack an enzyme that helps to produce material for the sugary coat. Another potential modifier on the scene!

https://pubmed.ncbi.nlm.nih.gov/35554935/

A Danish group (Rasmussen et al., 2022 J Neurochem) tested a new gene therapy approach for NPC2 deficiency, which could also be useful for ASMD – the keyword here is enzyme replacement. The blood-brain barrier prevents the entry of large molecules, viruses and other junk into the brain. Thank god! On the other hand, this barrier prevents treatments with anything that is too big to pass such as NPC2 or ASM. The barrier is made by a specific cell type, called endothelial cells, which form the walls of blood vessels. Processes of astrocytes and pericytes snuggle up to endothelial cells. The barrier is formed by specific structures named "tight junctions" that seal the space between endothelial cells. Some viruses can cross the barrier (probably by passing through endothelial cells) and deliver the code for NPC1 to cells in the brain. They form the basis of the latest gene therapy approaches. However, this doesn't work for all animal species, it remains to be seen in humans. The idea of the group was to make endothelial cells produce NPC2, and to deliver it to the brain tissue. Obviously, this will work only for NPC2 and ASM, but not for NPC1, as only the former are soluble in water, secreted and able to float around. The group used a virus named AAV-BR1, which is known to target endothelial cells. The results show that the approach works at least in mice. After intravenous injection of viral particles, endothelial cells in the brain produced NPC2 albeit at low quantities. On the other hand, the colleagues found that depending on the brain region also neurons got infected and started to produce NPC2, which would help. Much remains to be done. Further studies should show whether the amounts of NPC2 suffice to slow down disease progression in NPC2-deficient mice.

Animal models (ASMD)

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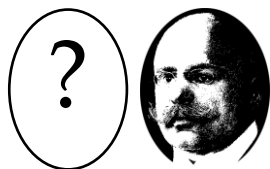
Similar topic: the group of Silvia Muro from Barcelona aims to get ASM across the blood-brain barrier (Solomon et al., 2022 J Control Release). A Troyan horse or piggyback approach couples ASM to specific molecules, mostly proteins, that are recognized by the endothelial cells mentioned above. After intravenous injection, this complex is taken up by endothelial cells on their blood-facing (for fans: apical) side and released on their brain-facing (for fans: basolateral) side. The whole thing is called transcytosis. The uptake, also known as endocytosis, can use different mechanisms – it's biology! Therefore different approaches have already been tested in ASM-deficient mice - albeit with limited success, probably because the amounts of ASM reaching the brain were low. The new study used mice, post-mortem brain samples of patients and cell cultures to address an important question, whether and how endothelial cells and their uptake capacity change during the course of the disease. The results show that in ASMD some uptake mechanisms work better, others break down. The new insight may help to choose the right piggyback enabling a smooth transcytosis.

https://pubmed.ncbi.nlm.nih.gov/35705808/

A publication in the prestigious journal *Nature* has nothing to do with ASMD, it's about the enzyme ASM and its role in cell death (Nozaki et al., 2022 Nature). The paper provides an opportunity to mention an important aspect. The enzyme is not only present in the lysosome, it can also be released by cells to work at the plasma membrane or in the extracellular space. There are numerous cellular processes, where the enzymatic cleavage of sphingomyelin to ceramide and phosphocholine plays an important role. They range from repair of the cell membrane, to tumor growth and controled cell death. So, there is something special about ASM as about NPC1 (and probably NPC2). These proteins are involved in processes that are independent from lysosomal recycling. The discovery of these functions in the 1990s (ASM: cell death etc.) and the 2010s (NPC1: ebola virus receptor) has boosted the respected research areas, and increased the number of publications that are not concerned with Niemann-Pick diseases.

https://pubmed.ncbi.nlm.nih.gov/36131016/

Another publication, again in *Nature* and again not related to ASMD or NPCD, should be mentioned. A group at Stanford University in California created a new tool to study lysosomes (Laqtom et al., 2022 Nature). A genetically modified mouse allows now to express in a cell type of interest (let's say neurons) a specific lysosomal protein. So what? The point here is that the protein was modified, so it can be used
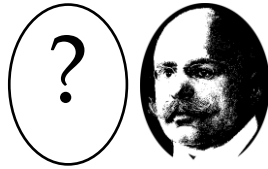
to pull out lysosomes from a sauce of cells. This allows to purify lysosomes from a specific cell type and to peek into their content with the clenched power of modern molecular analyses. Using the tool, the authors solved a long-standing mystery. They revealed the molecular culprit of a very rare lysosomal disorder named juvenile neuronal ceroid lipofuscidosis, also called Batten disease. The mutated gene, CNL3, was known, but not what it does. The study shows that the encoded protein called Battenin – ok, the name is not really inventive – shuffles glycerophosphodiesters out of the lysosome. These molecules originate from the cleavage of specific lipids and are to be recyclced. If battenin is broken, the stuff accumulates in lysosomes and causes havoc, similar to lipids in the case of ASMD and NPC. A nice example how new tools can resolve old problems. Hopefully, the new mice can also bring new insight in ASMD and NPCD.

Cell-based models (NPCD)

https://pubmed.ncbi.nlm.nih.gov/35508140/

A study of cultured cells (Tuck et al., 2022 Cell Rep) is worth to be mentioned, as it establishes a link between NPC1, cholesterol, and a small but famous-infamous protein called tau. Believe it or not, as of today there are 33,245 publications related to "tau protein". Why? The protein, whose full name is *microtubule-associated tau protein*, is attached to microtubuli. These long and highly dynamic "wires" are part of the scaffold along which lysosomes and other structures glide from A to B inside a cell. Some forms of tau protein clump easily and form neurofibrillary tangles – already heard about them? Probably yes, because these bundles emerge in a large number of neurodegenerative diseases called tauopathies. This includes NPCD, but also Alzheimer's disease; that's why so many publications! Whether these bundles contribute to the diseases is still unclear. Some say yes, some deny. Within the last years, it was found that the tau bundles can travel from one nerve cell to the next, and provoke the formation of new bundles. There are different theories how this works. The group found using all sorts of technical tricks that the cholesterol content of the cell membrane determines whether the tau stuff can enter cells: the lower – like in NPCD –, the easier the tau bundles can enter. Notably, the mechanism of entry depends on the type of cell. Stay tuned for more in case these tau protein dumplings are really harming nerve cells.

https://pubmed.ncbi.nlm.nih.gov/35653304/

A study from the realms of fundamental science brings new insight how NPC2 reaches its destination, the endosomal-lysosomal system (Anderson et al., 2022 Mol Biol Cell). Get this: at any given moment within a cell hundreds of proteins have to be produced and shuttled to their workplace. This also applies to NPC1, NPC2 and
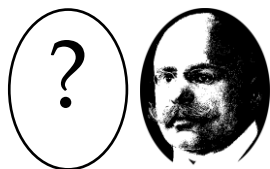
ASM requiring different mechanisms: one sits in the membrane, the others in the cellular juice. So far, these mechanisms are little understood. In brief, the authors show that a whole gang of proteins, for experts the BORC-ARL8-HOPS complex – easy to remember! – delivers NPC2 to the late endosome/lysosome. If one of these components fails, cells show similar cholesterol accumulation as provoked by NPC1 or NPC2 deficiency. A question is whether this complex could serve as drug target.

https://pubmed.ncbi.nlm.nih.gov/36009508/
A study from Italy (Capitini et al., 2022 Biomedicines) addresses the long-running search for ways to monitor pathologic lipid accumulation in lysosomal storage disorders. The authors used skin fibroblasts from patients and filipin, which binds to cholesterol, and choleratoxin B, which binds to GM1. The latter is a well-established tool to label cells, notably neurons. No worries, the tool is non-toxic as it comprises only the part that binds to cells ("subunit B"). The authors show that flow cytometry can be used instead of fluorescence microscopy to detect accumulation of cholesterol or GM1. For cytometry, cells are put in a watery suspension and chased through a machine that measures for each cell in the suspension selected parameters including fluorescence intensities. Although too cumbersome for diagnosis, the method could be used to measure effects of new drugs on patient fibroblasts. In fact, previous studies have shown that cytometry with other fluorescent dyes can be used in the context of NPCD. Still, the approach requires a machine that costs as much as a mid-size (German) car with all bells and whistles.

https://pubmed.ncbi.nlm.nih.gov/35929194/
There is news concerning therapeutic approaches. A group with Christian Grimm at the University of Munich (Germany) shows that activation of a lysosomal protein named two-pore channel 2 (TPC2) lowers pathologic lipid accumulation in NPC1-deficient cells (Scotto Rosato et al., 2022 EMBO Mol Med). As indicated by the name, TPC2 is an ion channel that can be activated by a small molecule imitating its natural ligand. Together with other channels and transporters, it regulates calcium concentration in the lysosome. This regulation is crushed by NPC1 deficiency. As stated in previous issues, calcium serves as an important signal in cells that triggers a whole range of cellular processes. One famous example is the release of neurotransmitter at synaptic connections between nerve cells. With respect to the lysosome, the release of calcium induces its fusion with the plasma membrane. Thereby, lysosomes throw their content out the door. Activation of TCP2 provokes this process, and enhances autophagy, the mechanism by which cells recycle wrecked organelles. Reactivation of both processes can reduce lipid accumulation in NPC1-deficient cells. The data-loaded publication shows efficacy of this approach in

several other storage diseases including mucolipidosis type IV und juvenile neuronal ceroid lipofuscidosis. The authors provide first tender hints that the approach works in animal models at least for mucolipidosis. The channel is a candidate for new therapeutic approaches, if experiments in NPCD animal models validate positive effects.

https://pubmed.ncbi.nlm.nih.gov/36125338/

Continuing chipperly with new therapeutic approaches: next stop messenger RNA. As a reminder, messenger RNA is generated in the nucleus by a process called transcription based on the genomic code contained in stretches of DNA. The strings are escorted out of the nucleus and hauled over to the endoplasmatic reticulum. There, proteins are synthesized in a process called translation according to instructions on the messenger RNA. Thus, messenger RNA forms the basis for gene therapies, if one can manage to introduce these large and largely unstable molecules in cells. There are several possibilities, one is transfer by a virus like the famous adenovirus-associated virus (AAV), which is tested for NPCD. Alternatively, messenger RNA can be shuttled into cells when bound to specific chemical vehicles. The results of an Australian team obtained with patient-derived skin fibroblasts suggest that RNA-based therapy may work without virus (Furtado et al., 2022 Mol Pharm). A prime advance was the optimization of RNA in terms of stability and translation efficacy meaning the efficacy with which the protein is synthesized. The first results show that the RNA allows fibroblasts to produce normal NPC1, which reduces cholesterol accumulation. A question is whether this works in animals.

Cell-based models (ASMD)

https://pubmed.ncbi.nlm.nih.gov/35727525/

A group from Milano in Italy introduces a modified cell culture model for the infantile neurovisceral form of ASMD, also known as Niemann-Pick Typ A (Carsana et al., 2022 J Mol Neurosci). To this end, patient-derived skin fibroblasts were cultured for several weeks and fed with sphingomyelin, which accumulates in ASM-deficient cells. The study shows that this long-term treatment worsens the pathologic changes in cells, it impairs mitochondrial function and creates radicals that vandalize their interior. Further studies will show whether the new protocol will provide new insight in this severe form of ASMD.

https://pubmed.ncbi.nlm.nih.gov/36071159/

Here's another publication - again in Nature - from the department "Has nothing to do with Niemann-Pick, but is important anyway" authored by a duo from Pittsburgh (Tan & Finkel, 2022 Nature). The topic is repair of damaged lysosomes. Indeed, cells
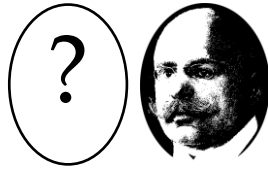
can repair minor defects in lysosomes (and other organelles) "do-it-yourself". They do not even need any of those home-improvement stores. Previous work provided first hints how cells do it, but much remains to be learnt. The two authors uncovered a new mechanism by coursing through the fields of molecular biology. In brief, they first uncovered a tag for leaky lysosomes named phosphatidylinositol-4-phosphate. This attracts a quartett of proteins that belong to the family of oxysterol-binding protein (OSBP)-related proteins. These in turn induce contacts between broken lysosomes and the endoplasmic reticulum, where lipids including cholesterol are manufactured. These contacts seem to allow the transfer of material to fill the holes. In addition, the repair process requires a protein named ATG2 that also contributes lipids for repair. Ok, if it were easy, we would know already. The new pathway could become a prime target for new therapeutic approaches for lysosomal diseases, once it has been validated by independent studies and once it is known, which cells use it.

Cell-based models (ASMD + NPCD)
https://pubmed.ncbi.nlm.nih.gov/36065186/
One more publication that introduces a new therapeutic target for NPCD and potentially ASMD (Chen et al., 2022 iScience). This is about a protein called tumor necrosis factor receptor-associated protein 1 or TRAP1, a.k.a. heat shock protein 75. It putters along in mitochondria, which are commonly called the cellular power plants. What it does there is not clear. The protein belongs to the large family of chaperones, so it probably helps other proteins to fold and function correctly. There are also hints that in nerve cells it hangs around outside mitochondria, but again its function is unknown.

The discovery of TRAP1 resembles one of those not necessarily rare saturday evenings, where one goes out with one person and wakes up next morning with another. In between, purely biological processes may have happened. The Ioannou group searched for molecules that activate a specific protein named Rab9. This was known to reverse lipid accumulation due to broken NPC1. The group performed one of those screens and caught two candidates, named ML405 and 1685. Further experiments showed that indeed ML405 reverts lipid accumulation in cell models for different lysosomal diseases including ASMD. However – back to Saturday night –, the target protein wasn't Rab9 but TRAP1. First experiments with NPCD mice showed a modest increase in lifespan. It remains to be seen whether these drug and target candidates will take off.

Miscellaneous (NPCD)

https://pubmed.ncbi.nlm.nih.gov/36154185/
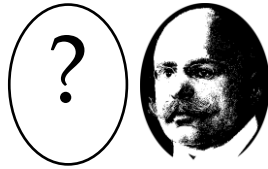
Attention melon, pumpkin/Halloween and cucumber lovers: Japanese colleagues report that the cucumber anthracnose fungus *Colletotrichum orbiculare* requires NPC1 and NPC2 to handle its needs similar to other cells with a real nucleus (Kodama et al., 2022 mBio). If either protein is missing, its plasma membrane goes bust, and it cannot enter into those plants.

https://pubmed.ncbi.nlm.nih.gov/36085278/

A study from the Platt group brings new insight to tuberculosis (Weng et al., 2022 Nat Commun). The disease is provoked by infection with *Mycobacterium tuberculosis*. Unfortunately, this bug can sort of hibernate in cells, as it manages to escape all defense mechanisms of the body. The question is still how. Early work of the group suggested that the bacterial cell wall contains molecules that inhibit the activity of NPC1. So what? Thereby, the bugs escape death by phagocytosis. This eating behavior of cells, notably macrophages, is messed up by NPC1 deficiency. The new work corroborates the hypothesis and shows that those bacterial strains that provoke tuberculosis, also inhibit NPC1 activity. Which molecules and how remains to be explored.