SCIENCE news

Selected articles featuring current research supported by the Novo Nordisk Foundation

MYSTERIES OF THE BRAIN

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How the ear picks up what we say

HORMONES AND METABOLISM

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FIGHTING CANCER

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DIET AND LIFESTYLE

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”The science of today is the technology of tomorrow.”

Edward Teller

100 fantastic research stories.

Two years ago, the Novo Nordisk Foundation decided to present some of the exciting stories behind the latest research that we have supported at universities and hospitals in Denmark. As a result, we set up a website, ScienceNews, and started posting these stories in both Danish and English. We quickly found that there is great interest in reading them – both in Denmark and abroad.

Here we have compiled a selection of our research stories from 2019. You do not need to browse very far in this publication to discover how fascinating and diverse current research is. The stories range from practical studies of new treatments for cancer or heart disease to basic research, the results of which can potentially improve people’s lives and the sustainability of society.

We hope that these stories provide an insight into researchers’ incredible level of commitment and give you a sense of how their quest for understanding the marvellous world of science and the human body can often be a long-term struggle. This commitment is driven by a desire to make a difference for people and societies around the world.

I hope you will enjoy reading the stories.

Birgitte Nauntofte
Novo Nordisk Foundation

Content

*This magazine is published in limited numbers and presents a wide range of research stories within **ten different research themes**. For each theme, there are six articles as well as samples of four additional articles, which you can find at **www.sciencenews.dk/en***

Fighting cancer

Content

See also the following articles at **sciencenews.dk**

Blood supply may be the achilles heel of tumours
Danish research shows that the arteries supplying blood to cancerous tissue act differently than other arteries in the body. Tumours may be targeted with medicine that alters blood flow thereby effectively killing them or making them more susceptible to radiation therapy.

Genetic imbalance in the immune system can lead to bile duct cancer
People with bile duct cancer or gallbladder cancer are often predisposed to genetic changes that make the immune system unable to kill the cancer cells. The discovery may lead to better diagnosis and treatment of people with these rare types of cancer.

Gold brings the dream of more targeted cancer drugs closer to realization
Danish researchers have shown that they can use gold nanoparticles to precisely target the delivery of medicine directly to where it is needed. In the future, gold nanoparticles may help in delivering more potent chemotherapy, for example.

New method reveals which cells resist chemotherapy
When cancer attacks the body, the body needs to combat the cancer cells while sparing the healthy ones. Conversely, cancer cells develop mechanisms to escape both the body's response to cancer and chemotherapy. The natural differences between humans and between tumours influence how effectively the body can fight cancer cells, including the response to chemotherapy. Researchers have now developed a method of differentiating between cancer cells and healthy cells – based solely on the composition of the proteins in cells. This method can also show how cells react to external stimuli such as chemotherapy.

Breast cancer defies new medicine and keeps spreading

One of the greatest challenges in the battle against cancer is preventing the cells from the primary tumour from spreading. For decades, researchers have investigated how to inhibit the chemical capacity of cancer cells to break down and squeeze through membranes and tissue structures such as breast tissue – but without success. The researchers now finally understand why their attempts were in vain. Cancer cells also have a second, previously undiscovered, physical method of migrating through tissue. The frontline of the battle is to disable both the chemical and physical mechanisms that cancer cells use.

By Morten Busch

Treating a person with cancer is often considerably more difficult if the cancer has spread. Metastasis is when cancer cells have escaped from the primary tumour and have pushed their way through the tissue and spread via the blood or lymphatic system. If this happens, the cancer can spread throughout the body, making the battle much harder to win. Some years ago, researchers successfully identified the metalloprotease enzymes cancer cells use to chemically degrade part of the tissue to enable the cells to migrate through it. However, years of effort in trying to prevent this have been unsuccessful. Now there is an explanation.

"We know that cancer cells have a chemical mechanism that helps them to break out of the tissue in which the tumour originated. We have now shown that breast cancer cells can also physically push themselves out of their original tissue and thus become invasive tumours without using the chemical mechanism. This means that simply inhibiting the chemical mechanism of cancer cells cannot stop their progress. We also need to prevent them from physically breaking through the tissue barriers," explains Ninna Struck Rossen, postdoctoral fellow at Biotech Research & Innovation Centre (BRIC), University of Copenhagen and co-author of the study carried out at Stanford University and published in Nature Communications.

Tiny stiff arms

The discovery is based on inventing a special combination gel that physically resembles the tissue in a woman's breast. This biologically active hydrogel comprises reconstituted extracellular matrix. By combining this hydrogel with alginate, another hydrogel derived from algae that is biologically inactive, the researchers created a structure that closely resembles breast tissue. In addition, the physical properties of the hydrogel can be changed without changing its chemical properties. In particular, the plasticity of the combination hydrogel can be regulated independently of how stiff it is.

"The alginate is not broken down by the chemical mechanism that cancer cells use to migrate through tissue. We were able to examine how the cancer cells, similar to migrating through tissue in the body, could still migrate through the combination gel. We thereby showed that the cells must have another mechanism for penetrating tissue. We also confirmed that the cancer cells retained their ability to penetrate the tissue, even when they were exposed to protease inhibitors that blocked the chemical mechanism."

The researchers used microscopy to follow how the cancer cells penetrated the tissue to try to determine how the cells could migrate through the hydrogel without using the chemical mechanism. The images obtained showed that invadopodia on the surface of the cancer cells assisted this migration.

"The process could be described as tiny stiff arms that push, create and expand small holes in the hydrogel. After a couple of hours, the cancer cells had created an opening that was big enough to enable them to squeeze through, and they used this method to gradually work their way through the tissue. Although the process was much more rapid when the cancer cells had both chemical and physical mechanisms, the physical mechanism was sufficient."

Stiff tissue can promote cancer

These new results may turn out to be a major breakthrough, because for decades researchers have tried to hinder metastasis by inhibiting the protease enzyme of cancer cells: the chemical mechanism for breaking down tissue. However, this strategy has been very disappointing. The new research explains not just why but also provides clues for possible future strategies.

"Protease inhibitors cannot be used alone but must be combined with equivalent mechanisms to combat the physical capacity of cancer cells such as the invadopodia. My colleagues at Stanford Medicine and Stanford Engineering are therefore actively developing a new strategy for blocking both the physical and chemical mechanisms cancer cells use to escape, which may be a new way to combat cancer."

Because the new hydrogel is so similar to human tissue, the experiments also revealed a previously unknown process. Cancer tissue is actually malleable. In addition, if the physical forces of the cancer cells influence the tissue, it can change and become stiff. This stiffness turns out to promote the spread of the cancer cells.

"Our research shows that cancer cells become more aggressive in stiff tissue and are therefore better at penetrating it. The way cancer cells physically affect tissue can also change it, increasing the likelihood that the cancer cells will migrate. So finding a method to keep this tissue soft – during cancer treatment – might also avoid metastasis."

"Finding a method to keep this tissue soft – during cancer treatment – might also avoid metastasis."

Ninna Struck Rossen

"Matrix mechanical plasticity regulates cancer cell migration through confining microenvironments" has been published in Nature Communications. In 2015, the Novo Nordisk Foundation awarded a visiting scholar fellowship at Stanford Bio-X to Ninna Struck Rossen, Biotech Research & Innovation Centre (BRIC), University of Copenhagen.

Danish health data:
People develop certain diseases before getting cancer

"A large-cohort, longitudinal study determines pre-cancer disease routes across different cancer types" has been published in Cancer Research. Jessica Hu is the first author. The study was a collaboration between researchers from the Novo Nordisk Foundation Center for Protein Research, University of Copenhagen and the Department of Infectious Diseases, Rigshospitalet, Copenhagen. In 2017, the Novo Nordisk Foundation awarded the Hagedorn Prize to Jens D. Lundgren, a main author.

Cancer often seems to appear out of the blue. Now the first study of its type shows the patterns of the other diseases people get before developing cancer. Cardiovascular diseases, obesity and genitourinary diseases are most common. The results can be used to better identify and screen the people with the highest risk and to search for new causes of cancer related to genes and lifestyles.

By Morten Busch

Global populations are ageing. Only a century ago, many people died young from infectious diseases, but as populations age, the disease pathways become more complicated, since many older people have several diseases at the same time. One disease that is increasing rapidly is cancer. To better predict who will develop cancer, Danish researchers analysed historical data from 6.9 million people to determine which diseases people get before developing cancer.

"For all types of cancer as a whole, people most frequently develop cardiovascular diseases, overweight and genitourinary diseases before developing cancer. Given this knowledge, we hope to become better at finding people at high risk of developing cancer and to find genetic links between the diseases to enable us to become better at preventing and detecting cancer early," explains Søren Brunak, Group Leader, Disease Systems Biology programme, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

"We had enough data to investigate 17 types of cancer and found clear disease patterns for seven of them."

Søren Brunak

Conclusions are premature

The study is the largest of its type in the world and has only been possible because of the Danish Cancer Registry, which contains data as far back as 1943. The study included data from 700,000 people with cancer. The researchers compared data from the Danish Cancer Registry and the Danish National Patient Registry – a national registry of the activity of all Danish hospitals – and found trends in the disease pathways.

"We had enough data to investigate 17 types of cancer and found clear disease patterns for seven of them. We already knew some of these patterns: for example, people tend to develop chronic obstructive pulmonary disease before they get lung cancer, but some disease pathways have been less prominent, such as many people developing such circulatory diseases as coronary artery spasms and ischaemic heart disease before developing cancer. This is important information that can potentially help doctors to focus early on the potential for these people to develop cancer."

Thus, the researchers found clear patterns in the diseases developing 10 years before people develop cancer of the breast, prostate, ovaries, lungs, skin and stomach and non-Hodgkin lymphoma, but even though the patterns are quite clear, the researchers emphasize caution in interpreting the patterns of earlier disease as being causally linked to developing cancer later.

"Although the disease pathways are rather obviously and notably associated with developing cancer, we cannot conclude that they are causally related. Just as we know that human papillomavirus (HPV) can lead to cervical cancer, we need to determine whether these diseases cause cancer, so the new results mainly give us ideas about where to look for associations."

Seeking the Achilles' heel of cancer

Inflammation is a factor the researchers generally speculate about in relating the early disease pathways to developing cancer later. Cardiovascular diseases, obesity and genitourinary diseases are associated with considerable and long-lasting inflammation if they are not treated.

"Inflammation is a possible and very relevant common denominator, so this is one question we will seek to answer in the years to come. We know that people have far fewer genes than previously thought: about 20,000. This probably means that the same genes can be associated with several diseases, so we are investigating these links."

In searching for genetic associations, the researchers are also focusing on non-oncogene addiction genes. Previously, cancer research has focused on oncogenes – genes that have the potential to cause cancer – but cancer researchers are increasingly seeking the genes that cancer cells need to survive.

"In our search for links between disease pathways and cancer, we especially focus on finding these genes, because they can prove to be the Achilles' heel of cancer, which could perhaps be targeted using medicine. While we clarify these links, the results should also be used to focus on the people with these characteristic disease pathways to target those who should be screened regularly."

Cancer treatment could soon target a protein

Researchers have found a protein that is important in helping tumours to survive, grow and metastasize. The protein could become a new target for cancer treatment.

By Kristian Sjøgren

Cancer treatment should not always target the cancer cells alone. This is the conclusion of new research that suggests that the support cells surrounding the tumour may also be a suitable target in combating cancer.

One of these potential targets may include the protein nicotinamide N-methyltransferase (NNMT), a metabolic enzyme that supports tumour growth and the ability to metastasize.

NNMT may therefore also become a treatment target because, if researchers can reduce the expression or activity of this cancer-promoting protein, they may also slow down tumour growth to give other treatments time to cure the person with cancer.

At least this what a researcher behind the discovery hopes.

"For a long time, researchers have almost exclusively focused on the cancer cells to understand cancer and find ways to treat people with the disease. But a tumour is a complex entity with many other cell types involved – collectively called the tumour stroma or microenvironment – that are by definition non-cancerous. We have investigated some of these support cells to determine how they behave during metastasis and discovered a common protein pattern in these cells that new treatments can target," says Fabian Coscia, postdoctoral fellow, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

The new research results were recently published in *Nature*.

Cancer needs help to proliferate
Cancer cells depend on support to proliferate and metastasize.

They need help from many different support cells that they reprogramme to assist their aggressive growth.

One way cancer cells do this is by inducing nearby cells to send them nutrients or growth signals or to create space in the adjacent tissue so that the cancer cells can divide or metastasize.

Fibroblasts are one type of support cell that are present throughout the body. Under normal conditions, they fulfil important physiological tasks during wound healing, for example.

"However, fibroblasts near cancer cells promote tumour growth because the cancer cells induce the fibroblast cells to act on their behalf. This is interesting that cancer cells can communicate with their surroundings and make them work for rather than against them, but this clearly also provides some therapeutic opportunities," says Fabian Coscia

Similar protein profiles among all cancer cases
Fabian Coscia and colleagues examined more than 5000 proteins in the tumour and tumour stroma (support cells) from normal and cancer tissue to see whether any behaved differently in the two groups.

The study is the first to systematically compare the protein profiles of the tumour stroma during ovarian cancer metastasis.

The most interesting discovery is that the protein profiles of the metastatic stroma were highly similar across tumours from different women with advanced-stage ovarian cancer, which was the opposite in the cancer cell compartment.

"Cancer cells seldom have common characteristics in the expression of proteins, which also creates difficulty in identifying common targets for treatments across the people with cancer. It is therefore interesting that we found a common signature in the support cells, because then general treatments could be developed," says Fabian Coscia.

Proteins induce cells to work for cancer cells
The researchers focused on one specific metabolic regulation protein that is extremely active in reprogrammed fibroblasts in cancer: cancer-associated fibroblasts.

NNMT causes several changes in the gene expression and metabolism of fibroblasts, and when the activity of NNMT changes, the fibroblast changes from a normal fibroblast to a cancer-associated fibroblast that supports cancer growth.

Overexpression of NNMT leads to increased cell division, growth and metastasis in the related cancer cells.

"NNMT regulates the tumour-promoting effect of fibroblasts," says Fabian Coscia.

A novel NNMT inhibitor
In further studies, the researchers used a novel NNMT inhibitor (NNMTi) that was then tested to inhibit cancer growth and metastasis in pre-clinical models.

The idea was that, if they suppressed the activity of NNMT, the cancer-associated fibroblasts would revert to their normal state of not supporting tumour growth.

The researchers tested NNMTi on fibroblasts, and it worked well in inhibiting cancer cell growth.

"We tested NNMTi in pre-clinical mouse models and it worked. It slowed down the ability of cancer cells to metastasize, so the cancer did not progress as rapidly as it might otherwise," says Fabian Coscia.

A new type of treatment?
Fabian Coscia thinks that the discovery could be the mechanistic basis for a new class of cancer treatment in the future.

The vast majority of current treatments focus on attacking the cancer cells, such as standard chemotherapy. However, cancer cells often acquire drug resistance so that the same drug cannot be used again. It is therefore important to identify alternative treatment regimens that have a different mode of action.

If researchers can use NNMTi to keep cancer cells from taking control of the other cells, doctors can attack cancer in several ways.

"We hope that one day we will be able to treat people with combination therapy – for example, chemotherapy in parallel with an NNMT inhibitor – so the nearby tissue no longer works for the tumour but even fights it. Many treatments could become much more effective if they do not have to fight the effects of the support cells that strengthen the tumour," says Fabian Coscia.

"Proteomics reveals NNMT as a master metabolic regulator of cancer-associated fibroblasts" has been published in Nature. Several authors are employed in the Clinical Proteomics Group, Proteomics Program, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

Pregnancy associated with protection against breast cancer

New Danish research shows that giving birth to children can significantly reduce women's risk of breast cancer. However, the stage in the pregnancy at which the woman gives birth matters. If the baby is born before week 34 of the pregnancy, the woman is not protected against breast cancer, whereas a longer pregnancy protects. Giving birth to more than one child also improves the protection, and women older than 28 years who have children do not achieve protection.

By Kristian Sjøgren

Worried about breast cancer?

New Danish research suggests that having children at a young age is associated with a markedly reduced risk of breast cancer later in life.

The researchers obtained information from 2.3 million women in Denmark and 1.6 million women in Norway, and the conclusion is clear: every pregnancy reduces the risk of breast cancer later in life by 8–9%.

"It has long been known that giving birth is associated with a lifelong reduction in the risk of breast cancer. This study went a step further and can now determine how long women must be pregnant before achieving protection," says the researcher behind the new study, Mads Melbye, Professor, Statens Serum Institut, Copenhagen, Denmark.

The research results, in which PhD student Anders Husby is the first author, were recently published in *Nature Communications*.

Pregnant women protected after 33 weeks of pregnancy

The researchers obtained data from various Danish registries, including cancer registries, birth registries and abortion registries. Using these data, they identified the extent to which being pregnant reduced the risk of breast cancer and when in the pregnancy the protective effect occurs.

Previously, researchers used data from the Danish abortion registry to show that being pregnant for 8–12 weeks and then getting an abortion does not protect against breast cancer. A longer pregnancy is needed before the protective effect occurs, and the researchers wanted to determine how long the pregnancy should last to provide protection.

The new results show that the protective effect only occurs after week 33 of the pregnancy. Women who give birth up until week 33 of pregnancy do not achieve protection, whereas women who give birth during week 34 of pregnancy or later obtain protection.

In addition, the results show very clearly that this effect does not increase slowly but changes very abruptly, with the cut-off between weeks 33 and 34 of pregnancy.

"The Danish registries are renowned globally because they go way back in time, are very complete and cover the entire population. We used the registries to very clearly determine when the protective effect occurs. We also found that each additional child increases the protective effect by about 8–9%. This means that the more children a woman gives birth to, the better she is protected against breast cancer for the rest of her life," Mads Melbye explains.

Translating new knowledge into preventive treatment

Mads Melbye cannot yet explain why pregnancy and giving birth are associated with protection against breast cancer.

He says that the cells in the breasts undergo major changes in pregnancy, and towards the end of pregnancy many breast cells mature for the purpose of producing milk.

Breastfeeding itself is not the explanation, because the researchers found the same protective effect among women who experienced stillbirth.

The researchers are currently investigating the composition of several thousand substances in women's blood in weeks 33 and 34 of pregnancy to determine whether this composition changes and might help to explain the protective effect.

Mads Melbye imagines that some substances are released into the blood at this specific time in pregnancy and help to mature and thus also protect the breast cells.

"If we can determine which substances provide this protective effect, we could clearly convert them into preventive treatment as medicine. This is the ideal scenario in the long term," says Mads Melbye.

Protection ends after the mother reaches 28 years

The new research findings show that women should preferably be young for a pregnancy to achieve the protective effect.

Women who give birth when they are younger than 28 years old achieve the full effect of having been pregnant for more than 33 weeks, whereas a pregnancy after 28 years old does not produce any effect.

Again, the researchers face a question that they cannot yet answer, but the result is worth noting.

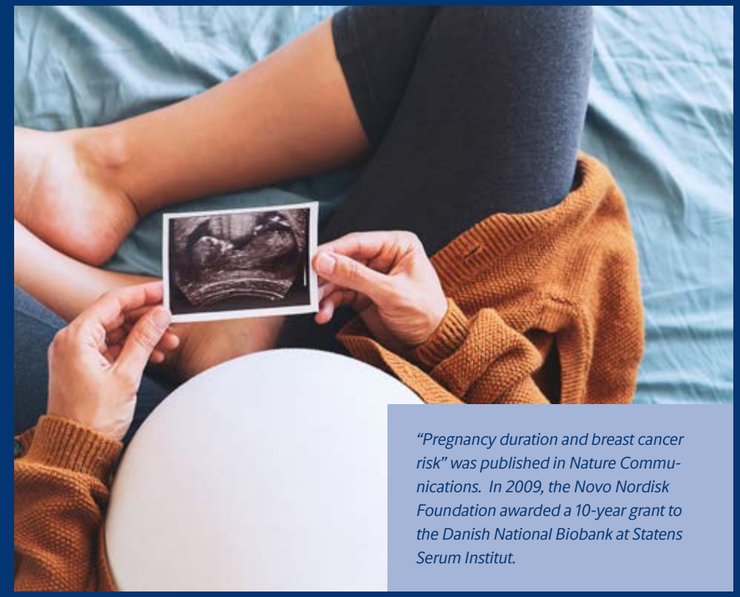
"It is interesting that, in Denmark, as women giving birth for the first time become older and older, the number of women with breast cancer has increased. These two trends may be linked," according to Mads Melbye.

Identical results in Norway

To ensure that the findings do not solely apply to women in Denmark, the researchers confirmed their results by analysing the data from Norwegian databases. The results were not merely similar to those from Denmark but identical.

The women in Norway also achieved a protective effect against breast cancer if they were pregnant for more than 33 weeks.

"This confirmed our findings and shows that our results are very solid," says Mads Melbye.



"Pregnancy duration and breast cancer risk" was published in Nature Communications. In 2009, the Novo Nordisk Foundation awarded a 10-year grant to the Danish National Biobank at Statens Serum Institut.

The body's final line of defence on the route to cancer

Each of the body's millions of cells contains an exact copy of our DNA. This places great demands on the machinery that replicates the DNA when cells divide. If things go wrong serious diseases such as cancer can emerge. Now scientists have found what seems to be the final chance for cell division that may prevent daughter cells from inheriting permanent DNA errors from the mother cell. The new finding provides important knowledge on what can go wrong when cancer develops and reveals an important Achilles heel in cancer cells.

By Morten Busch

Damage to our genes is a frequent cause of cancer. This damage eliminates the cells' ability to stop dividing. Researchers already know a lot about how tobacco smoke and ultraviolet radiation damage our DNA and lead to lung or skin cancer, but they know very little about how cancer can develop naturally from errors in the continual cell division in the human body.

"These errors occur all the time when the body replicates its DNA through cell division. We have now discovered how the body ensures that this type of DNA error is not propagated. During replication, the cell engulfs and protects the damaged DNA while simultaneously restraining replication. The new knowledge of how cells prevent damage to the genetic code may be crucial for improving existing treatments and developing new treatments against cancer," explains Kai John Neelsen, senior scientist in Jiri Lukas' group at the Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

Cell division on standby

The new finding builds on a discovery 8 years ago when Jiri Lukas and his group found that our tissues defend against errors during cell division through highly specialized organelles (structures) within the cell nuclei: 53BP1 (p53 binding protein 1) nuclear bodies. Despite small in size, these organelles control the cells' growth rate and play a significant role when the cells divide.

"It all started when we observed 53BP1 nuclear bodies as peculiar globular structures that often emerged right after cell division in the newly born daughter cells. We did not understand the purpose of this phenomenon, but since then we have followed the 53BP1 nuclear bodies during cell division by marking them with fluorescent dyes and observing with microscopes in living cells. When the daughter cell is born, the special protein structure containing the 53BP1 protein immediately engulfs any DNA damage inherited from the mother cells and sets in motion a series of events whose ultimate goal is to mend the damage and thus stop its propagation to the next generation of cells."

The 53BP1 nuclear body in the daughter cell thus gives the body an extra and last chance to repair the damaged DNA that the mother cell could not repair itself. In addition to identifying and engulfing the DNA errors, the cell machinery does another remarkable thing: putting cell division on standby, so the error can be corrected, and the researchers found out how in the new study.

"The 53BP1 nuclear body restrains and adjusts the replication timing process so that the proteins can repair the DNA lesions at exactly the right time. It gradually becomes smaller and disappears at exactly at the time when the cells are ready to repair the damage. We have also shown that, if this last chance fails, the error can no longer be corrected and will probably lead to serious diseases, including cancer," summarizes Julian Spies, first author.

Improving existing cancer treatments

The repair protein in the cells thus has extra time to repair the errors in the genetic code, and the researchers have now also found the RAD52 enzyme that actually carries out the repair. According to the researchers, RAD52 should now be included as a key component of the arsenal of tumour-suppressing proteins that can be used in fighting cancer.

"It may seem somewhat strange that RAD52, which is actually repairing cells, can be a target for cancer treatment. However, remember that cancer cells divide more rapidly and therefore need RAD52 more than other cells. So, if you can suppress RAD52, you can strike cancer cells with extra impact."

The researchers do not yet know enough about RAD52 and how it is regulated. If they find this out, they hope to be able to build a solid platform for developing both better and completely new drugs. The new discovery therefore greatly improves the understanding of how the body protects itself against many forms of cancer. This may be crucial for improving existing cancer treatments.

"An adult's body contains trillions of cells. Every day, a quarter of a trillion cells divide to rebuild or renew damaged or old tissue. Our findings provide better understanding of the natural timing of cell division and how the body defends against DNA damage and mutations. Using this knowledge, we can now begin to minimize the side-effects of current cancer treatments", explains Jiri Lukas his team's effort.

"53BP1 nuclear bodies enforce replication timing at under-replicated DNA to limit heritable DNA damage" has been published in Nature Cell Biology. The research was carried out at the Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

New blood test detects cancer early

Danish researchers and international collaborators have developed a method for detecting all types of cancer in the early stages through a blood test. This method may eventually save many people's lives.

By Kristian Sjøgren

Imagine that doctors could use a simple blood test to tell you not only whether you have cancer or not but also, if you do have cancer, where it is located.

This may sound like wishful thinking, but this dream is actually not as fanciful as it may seem.

Danish researchers have developed a method that enables researchers and doctors to diagnose all types of cancer very accurately by using a blood test.

"If we can achieve this, it will be quite revolutionary and will mean that we can detect cancer before the disease progresses to a stage at which treatment becomes difficult. We can also identify where the cancer is located. Both identifying that a person has cancer and its location are vital in hospital investigations," says a Danish contributor to the study, Claus Lindbjerg Andersen, Professor, Department of Clinical Medicine, Aarhus University and Department of Molecular Medicine, Aarhus University Hospital.

The Danish researchers and colleagues from the Netherlands and the United States recently published their results in *Nature*.

Cancer cells leave unique DNA fragments in the blood

The method is based on the fact that different types of cancer leave different DNA signatures in the blood.

The blood vessels in cancerous tissue often leak, which means that DNA from fragmented cancer cells can enter the bloodstream and thus circulate in locations where it should not normally be.

This means that the blood suddenly contains fragments of DNA from, for example, lung cancer cells, liver cancer cells or prostate cancer cells.

Further, DNA is folded differently in different types of cancer cells, depending on where the cancer is located. The researchers can measure these differences through a blood test to detect not only whether people have cancer but, if they do, what type of cancer it is.

"Our study is a proof-of-principle study in which we have demonstrated that a blood test can detect cancer," says Claus Lindbjerg Andersen.

Identifies cancer in 73% of cases

The researchers used their method to analyse the DNA fragmentation profiles of 236 people with breast, colorectal, lung, ovarian, pancreatic, gastric or bile duct cancer. The blood test detected cancer in 73% of the cases.

In addition, the method differed in how effectively it could identify the different types of cancer.

For example, the blood test detected only 57% of breast cancer cases but more than 99% of lung cancer cases.

"The current sensitivity of our method means that some individuals will have insufficient cancer cell DNA in their blood so that we cannot detect it in the amount of blood we examine," explains Claus Lindbjerg Andersen.

However, Claus Lindbjerg Andersen also says that the researchers are constantly trying to refine the method to detect cancer more accurately.

"The goal is to use blood tests to monitor people with cancer so that we intervene with treatment as soon as we see signs of the disease returning."

Claus Lindbjerg Andersen

Eliminating false-positive results

The researchers also tested the method on 245 healthy individuals and identified four as having cancer, even though they did not: false-positive results.

Eliminating false-positive results is important for the future of this method because there should be very few if the healthcare system is to screen people for cancer though blood tests on a large scale. This especially applies to screening older people for cancer, since they often have high blood pressure, diabetes and other conditions that may, but hopefully will not, affect the outcome of the blood tests.

The researchers are therefore testing their method on both older people and people with various lifestyle-related diseases to ensure that ageing and diseases other than cancer do not affect the results of the blood tests.

"We do not expect that ageing and other diseases than cancer will affect our test results, but we have to exclude this possibility before we can proceed with developing our method. We need to know that being older and having other diseases do not do anything to the body that may be mistakenly identified as cancer in our test," explains Claus Lindbjerg Andersen.

Monitoring people with cancer after treatment

In addition to minimizing the risk of false-positive results, the researchers are also conducting experiments that test various applications for their cancer screening.

In particular, Claus Lindbjerg Andersen suggests that the blood test may be useful after cancer treatment.

After someone has been treated for cancer, doctors currently do not have many good methods to determine whether the cancer has disappeared completely or whether some has survived and is just waiting to grow again.

The major problem is that the survival rate for individuals whose cancer has returned is less than 10% over 5 years. A common reason is that the cancer is not detected early enough to treat it effectively.

Thus, one can imagine using the new blood test to continually monitor whether people's cancer returns. The researchers are conducting a new clinical trial to examine the effectiveness of this strategy.

"The goal is to use blood tests to monitor people with cancer so that we intervene with treatment as soon as we see signs of the disease returning. This will save many lives, because today we are not very good at finding the residual disease early enough for us to start effective treatment," says Claus Lindbjerg Andersen.

"Genome-wide cell-free DNA fragmentation in patients with cancer" has been published in Nature. In 2017, the Novo Nordisk Foundation awarded a grant to a co-author, Claus Lindbjerg Andersen, for the project Implementing Non-invasive Circulating Tumour DNA Analysis to Optimize the Operative and Postoperative Treatment of Patients with Colorectal Cancer.

Mysteries of the brain

Content

See also the following articles at **sciencenews.dk**

Different regions of the brain do not produce the same amount of energy
Researchers have determined how much energy is produced in two regions of the brain, each of which plays important roles in the development of diseases, including Alzheimer's disease, Huntington's disease and Parkinson's disease. The results show that these brain regions do not produce the same amount of energy, and this may help to provide greater insight into how the diseases develop.

How patients perceive mechanical restraint in forensic psychiatry
Denmark has committed to limiting the mechanical restraint of patients in forensic psychiatry. Fulfilling this ambition also requires understanding how these patients perceive the use of mechanical restraint in forensic psychiatry. Researchers have now asked patients about this.

Permanent night workers develop dementia more frequently
Permanent night workers frequently have disturbed and insufficient sleep. Nevertheless, it is still unknown how night work affects the brain in the long term. Now research shows that permanent night workers more frequently develop dementia. Whether this results from disturbed sleep, changes in health behaviour or other factors is too early to say. The researchers believe that both employees and the employer can act to mitigate the potential health effects of night work.

What happens when proteins are defective?
Danish researchers have linked changes in DNA to defects in proteins. This is a way to understand precisely how tiny variations in the body's DNA can result in diseases such as cancer, Alzheimer's disease, cystic fibrosis and phenylketonuria.

Researchers have found a key to fighting degenerative diseases

"Retinal and optic nerve degeneration in liver X receptor β knockout mice" has been published in Proceedings of the National Academy of Sciences of the United States of America. In 2016, the Novo Nordisk Foundation awarded a grant to Jan-Åke Gustafsson for the project Multiple Functions of Oxysterol Receptors in Modulation of Neurodegeneration.

People with cancer or infections are often attacked quickly and aggressively, whereas people with neurodegenerative diseases deteriorate slowly. Nevertheless, this slow decline of body and mind is almost unavoidable since these diseases are usually incurable. Researchers have now discovered what they describe as a key to preventing and curing neurodegenerative diseases such as multiple sclerosis and Parkinson's disease.

By Morten Busch

We have become increasingly better at treating people with acute diseases, but the number of people with chronic diseases has grown. Consequently, the hunt has intensified to stop diseases in which people's condition deteriorates continually over many years. New results show that some of these diseases can potentially be stopped – especially degenerative diseases of the central nervous system (neurodegenerative). The key is a signal receptor called liver X receptor β (LXRβ).

"Contrary to what the name might suggest, these receptors are present throughout the body, but we are specifically investigating their role as signal receptors for hormones and fatty acids in the nucleus of brain cells. LXRβ seems to play a key role in various neurodegenerative diseases. Influencing the receptor in specific ways appears to trigger protection against these diseases, which are caused by excessive activity in the brain's immune system, such as Parkinson's disease, multiple sclerosis and optic neuritis," explains a main author, Jan-Åke Gustafsson, Professor, Karolinska Institutet, Stockholm, Sweden.

Water channels malfunction

Liver X receptors (LXRs) expressed in the brain play a key role in maintaining cerebrospinal fluid and the health of neurons, including those that produce dopamine. These are the neurons that are destroyed in Parkinson's disease. In their latest experiments, the researchers focused on the role of LXRs in the retina.

"The retina is an extension of the brain. Like the brain, the retina degenerates naturally with age, causing several retinal diseases, including optic neuritis, calcification, macular degeneration and glaucoma. In our new experiments, we found that the LXRs are expressed in the retina and optic nerve and that a loss of LXRβ leads to a loss of retinal ganglion cells, which retrieve information from the photosensitive cells."

The experiments involved removing the gene encoding LXRβ in mice, causing them to lose ganglion cells in their eyes. Using a staining technique, the researchers discovered the mechanism: the mice were lacking aquaporin 4, an important protein in the cell membranes.

"The experiments clearly showed that losing the LXRs initiates the degeneration of the optic nerve. This confirms once again that LXRβ is a promising target for treating people with neurodegenerative diseases and, in this case, specific retinal degenerative diseases. These include both eye diseases arising independently and ones resulting from such chronic diseases as multiple sclerosis and diabetes," explains a co-author, Margaret Warner, Department of Biology and Biochemistry at the University of Houston.

Enormous untapped potential

The new research is specifically interesting in explaining how degenerative eye disorders such as optic neuritis develop. Indeed, much research suggests that understanding optic neuropathy may be the key to more broadly understanding how neurodegenerative diseases generally develop – which may ultimately lead to new therapies to treat people with Alzheimer's or Parkinson's.

"A genetic loss of LXRβ in mice increases the amyloid plaques known in neurodegenerative diseases, and LXRβ is therefore a potential therapeutic target for the diseases caused by excessive activity in the brain's immune system. This applies to Parkinson's disease, amyotrophic lateral sclerosis and multiple sclerosis."

"Influencing the receptor in specific ways appears to trigger protection against these diseases."

Jan-Åke Gustafsson

Previous experiments in mice have also confirmed the enormous potential of substances that can activate LXRs. These have successfully reduced the symptoms of Alzheimer's disease and lowered cholesterol levels in insulin-resistant mice, thus inhibiting the development of arteriosclerosis and lowering blood glucose. These substances have even been shown to suppress the spread of prostate cancer and breast cancer in mice.

"The effects of LXRs have only been shown in mice, so we do not yet know whether LXRs will benefit people. One major challenge in mice has also been that the side-effects of the potential drugs increase triglycerides in the blood. Efforts are therefore being made to develop new substances without these undesirable side-effects, so these substances can hopefully be used safely in treating people."

People with ADHD have restless legs syndrome more often

Restless legs syndrome is a neglected and very common condition that has not garnered much attention from doctors so far. However, it should, because it is associated with poorer quality of life and an increased risk of many diseases. Researchers have now found that restless legs syndrome is also associated with the risk of having attention-deficit hyperactivity disorder (ADHD).

By Kristian Sjøgren

”Restless legs syndrome can lower the quality of life of people who are severely affected, and unfortunately it more often afflicts people who already have a hard life.”

Henrik Ullum

You may be one of the people who tosses and turns for hours at night with your legs continually and involuntarily moving.

About 5% of Danes have restless legs syndrome, and getting out of bed and standing up every single night to make their legs calm down can be a torment and a real hassle. It can ruin their sleep, and disturbed sleep is associated with increased risk of many diseases, including cardiovascular disease, obesity and mental disorders such as depression. So restless legs syndrome should be taken seriously.

New Danish research now shows another reason to take it seriously, because it is associated with ADHD.

“Restless legs syndrome can lower the quality of life of people who are severely affected, and unfortunately it more often afflicts people who already have a hard life. Now we have found that restless legs syndrome is associated with ADHD, and this is important when doctors encounter symptoms of either condition. Perhaps it would make sense for doctors to investigate whether people with ADHD also have restless legs syndrome and disturbed sleep at night and whether people with restless legs syndrome also have ADHD,” explains the researcher behind the new study, Henrik Ullum, Clinical Professor and Chief Physician, Department of Clinical Immunology, Rigshospitalet, Copenhagen.

The new study has been published in *Sleep Medicine*.

Restless legs syndrome is a neglected condition with major effects

Restless legs syndrome is insufficiently studied and affects many people, but doctors currently do not know what to do about it.

Several known factors are associated with an increased risk of having restless legs syndrome: iron deficiency, smoking, overweight, low educational level and a generally unhealthy lifestyle.

People who have restless legs syndrome often experience involuntary movements and abnormal unpleasant feelings in their legs just before sleeping, but the syndrome may sometimes continue during sleep, and the involuntary movement can wake people up at night.

Although many people have restless legs syndrome and its various negative effects, treatment is not very effective or consistent. No medicine is effective, and many people try various self-care measures to ease the condition, such as adopting a healthier lifestyle or taking mineral supplements with iron.

Henrik Ullum hopes to change this.

“Our research has two distinct focus points. We focus on restless legs syndrome, because it is associated with iron deficiency, and in our blood bank we often find that blood donors get restless legs syndrome, because giving blood often leads to iron deficiency. We want to help them by providing iron to the ones who need it. Second, we focus on ADHD because it is becoming much more common,” he explains.

25,336 Danes participating

In the study, the researchers searched for associations between ADHD and restless legs syndrome.

They used data from the Danish Blood Donor Study, which included people who donated blood in Denmark from 1 May 2015 to 1 February 2017. The 25,336 participants completed two questionnaires: one on symptoms of restless legs syndrome and one on symptoms of ADHD.

The participants also provided information on sex, age, body mass index, smoking status, alcohol consumption, whole-blood donation history and self-appraised quality of sleep.

Of the 25,336 participants, 1322 (5.2%) were classified as having restless legs syndrome, and 653 (2.6%) experienced ADHD symptoms.

The researchers examined whether ADHD is associated with restless legs syndrome and found that people with ADHD had a 3.5-fold greater risk of having restless legs syndrome than the rest of the study group.

“These participants were not necessarily clinically diagnosed with ADHD, but their ADHD score indicated that they could be diagnosed with ADHD if examined,” says Henrik Ullum.

Doctors should offer guidance to people with restless legs syndrome

According to Henrik Ullum, the results should be used to focus more intensely on restless legs syndrome and its association with ADHD.

Specifically, he recommends that doctors ask about restless legs if a person has ADHD and pay extra attention to signs of ADHD if a person has restless legs.

For patients who have ADHD and restless legs syndrome, doctors should enquire further about lifestyle and at least ensure that the patients get plenty of iron. Treatment for restless legs syndrome may start by getting the patient to stop smoking, lose weight or eat more healthily.

“These are the options we have right now. More research and better treatment are needed before we can do more, but we hope this will be possible because having restless legs syndrome can be serious if it disturbs people’s sleep,” says Henrik Ullum.

“Self-reported restless legs syndrome and involuntary leg movements during sleep are associated with symptoms of attention deficit hyperactivity disorder” has been published in Sleep Medicine. A researcher from the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen contributed to the study and is a co-author.

Curing brain cancer by heating the tumours

For years, doctors have unsuccessfully battled the most malignant form of brain cancer: glioblastoma. A major problem is that the tumours can be hidden and the immune system detects them too late. Researchers have now succeeded in "heating" these immunologically "cold" tumours to fight them. Their first trial was so promising that they are preparing Phase 2 trials for developing immunotherapy. This method may prove to be even more effective in combating other types of cancer.

By Morten Busch

People with glioblastoma, a type of malignant brain cancer, have a poor prognosis today. They survive less than 18 months on average after diagnosis. Removing the tumour is insufficient, because the disease has already spread throughout the brain. Unfortunately, both these people's immune systems and doctors have difficulty in detecting cancer cells, and attempts to combat the disease so far have been unsuccessful. A large international research consortium has now taken a decisive step towards achieving a breakthrough.

"We have decided to abandon the current principles guiding treatment. Instead of just treating the tumour itself, we try to treat the whole brain. We do this by adapting personalized treatment precisely to each person's tumour. By targeting the treatment to the specific antigens in each person, we change the cancer from being immunologically "cold" to "warm" so that we can combat it with immunotherapy," explains Hans Skovgaard Poulsen, Associate Research Professor and Chief Physician, Section for Neuro-oncology, Department of Radiation Biology, Rigshospitalet, Copenhagen.

Changing from cold to warm

The results of the Phase 1 trials conducted based on the new treatment principles are so promising that they have been published in Nature. The new type of treatment has two phases. First, doctors use tissue samples to identify some of the antigens (peptides or protein fragments) previous studies have shown are generally extensively expressed in brain tumours. Based on this, the first wave of treatment can include an immunotherapy cocktail called APVAC1 (actively personalized vaccine 1).

"This is broad-spectrum treatment, but the people treated with APVAC1 also get personalized treatment right away. Alongside this, we begin to monitor the most important changes in the antigens in each person's tumours. If we find them, we can start producing vaccines against the specific antigens involved in this person's cancer."

While the personalized vaccine is being prepared, taking about 1 month, the brain cancer is combated with the broad-spectrum immunotherapy. Then the second phase of the treatment – APVAC2 – starts.

"The antibodies in the second treatment phase specifically target the changes that have taken place in each person's tumour. The treatment therefore affects the cancer cells specifically and effectively and does not harm the person's normal cells."

The great challenge previously has been that the antigens in glioblastoma are expressed at a very low level. Creating an immune response that is powerful enough to effectively combat the cancer has therefore been difficult. Glioblastoma has therefore been considered "cold" immunologically, so previous attempts with immunotherapy have failed.

"We are only currently conducting Phase 1 trials, primarily examining whether the treatment is feasible and whether it is toxic. But we have already achieved a strong immune response, so we seem to have succeeded in making the "cold" tumours appear "warm" and are therefore very optimistic about the upcoming Phase 2 trials."

Attenuated poliovirus can help

It is still too early to predict whether applying this treatment of a fatal type of brain cancer affecting nearly 200,000 people worldwide will be successful in the future. For now, the personalized vaccines have only been tested on 15 people at Rigshospitalet in the Phase 1 trials. Cautious optimism is therefore still needed.

"Most people had a strong immune response, and some survived longer than expected. We cannot yet determine whether the positive effects are random. We need to test the vaccine on a sample of more people before we can be sure that the vaccine is actually causing the effects."

The research has been carried out as part of a large European Union consortium led from Germany that has been awarded about DKK 60 million in grants so far. The consortium is now planning Phase 2 trials on a much larger group of people in collaboration with Immatics, a pharmaceutical company headquartered in Germany, to determine whether the vaccine has the desired effectiveness. If this also succeeds, Phase 3 trials can be planned to compare this treatment with existing treatments for brain cancer.

"We have considerable work ahead of us before people can be treated. We are, however, optimists, because we achieve a substantial immune response, and we will probably be able to increase these effects through such means as programmed death-ligand 1 (PD-L1) inhibitors, which can further boost the human immune response. Phase 2 trials are also currently being conducted at Duke University in the United States, using the attenuated poliovirus to increase people's immune response to cancer. So we are very optimistic that the survival rate will increase significantly in the coming years."

"Actively personalized vaccination trial for newly diagnosed glioblastoma" has been published in Nature. A European Commission grant of DKK 45 million funded the study. In 2015, the Novo Nordisk Foundation awarded a grant to Hans Skovgaard Poulsen for the project Impact of Renin-angiotensin System Blockade on Clinical Outcome in Glioblastoma Patients.

New discovery: How the ear deciphers speech

New research shows how the inner ear transforms acoustic pressure waves and frequencies so that the brain can understand them as speech. The discovery paves the way for new diagnostic opportunities, which may also eventually help people to restore lost hearing.

By Kristian Sjøgren

A new study by researchers from Sweden, Denmark, the United States and India shows what happens when the inner ear transforms the multitude of frequencies and pressure waves in speech so that the brain can understand it and people can thereby understand what is being said.

The research shows that the hair cells in the inner ear actually distort the sound so that the brain can perceive it through meaningful electrical signals.

The discovery may pave the way for new diagnostic methods to identify the causes of hearing loss. Over time, the discovery may also be used to develop new treatments that can restore lost hearing without using hearing aids or implants.

"Today, we can measure hearing loss, but it is often difficult to tell whether the problem is in the sensory cells, the neurons or other cell types in the inner ear that support the function of the sensory cells. If we do not know what causes hearing loss, more effective treatments are difficult to find. Our discovery enables new diagnostic methods to determine the exact causes of hearing loss and thereby to start to develop new treatments," says Anders Fridberger, Professor and Head, Department of Clinical and Experimental Medicine, Linköping University.

The research results, which also include contributions from researchers from Danish companies Oticon and Interacoustics, were recently published in *Nature Communications*.

The brain uses electrical signals to understand speech

Understanding the new research results requires understanding what speech is.

Speech is sound that enters the ear as acoustic frequencies and pressure waves. Speech has a physical signature that can be observed, for example, by speaking into a microphone and recording the sound on a computer. The sound is visualized as numerous lines that rapidly rise and fall – similar to measuring earthquakes with a seismograph.

All the frequencies and the rapid changes in the computerized signature of the speech are called the fine structure of the sound, and researchers call the shape of these lines in their overall structure the acoustic envelope. The ear transforms this envelope into understandable signals that the brain can perceive as speech.

"We have known that the brain uses the acoustic envelope to understand what people are saying, but we have not known how the inner ear extracts information about the envelope," explains Anders Fridberger.

Cells converting sound into electrical signals

In the new study, the researchers conducted several studies on both mice and people, inserting electrodes into the inner ear to determine how various cells in the ear respond to speech.

The inner ear has many parts, including the cochlea, which contains very sensitive auditory hair cells. Sound entering the ear sets these hair cells in motion.

The results of the studies show that the inner ear distorts the sound before the brain processes it. One might think that distorting sound would be undesirable, but the results show that the ear must transform acoustic pressure waves and frequencies into electrical signals that the brain can understand.

The research also shows that the inner hair cells decode the acoustic envelope.

This discovery suggests that speech initiates a unique type of electrical signal in the brain that other sounds cannot.

Each hair cell has a sensitive ion channel that opens and closes. Speech activates these ion channels, the hair cells distort the sound and transform it into electrical signals that are transferred to the brain, which then deciphers the electrical signals as speech," explains Anders Fridberger.

Improving diagnosis of hearing loss

The discovery is an important contribution to understanding how the inner ear works.

The inner ear is encased in thick bone, making access difficult when studying injuries that may explain hearing loss.

However, this can start to change now.

"We believe our results will improve the diagnostic procedures for various types of hearing loss, and this is really needed. The immediate applications have not yet been developed, but we hope we can apply the discovery in practice soon," says Anders Fridberger.

Another researcher behind the study, Thomas Lunner, Senior Researcher at Oticon, says that the discovery can also improve how hearing aids are calibrated.

"So far, it has only been possible to investigate the state of the outer hair cells, such as in screening newborns. Our research can help to create the first method for diagnosing the state of the inner hair cells, which can potentially help us make better individualized hearing aids," says Thomas Lunner.

"A mechanoelectrical mechanism for detection of sound envelopes in the hearing organ" has been published in Nature Communications. In 2015, the Novo Nordisk Foundation awarded a grant to Anders Fridberger for the project Clinical Testing of a New Strategy for Treating Hearing Loss.

Herpes increases the risk of mental disorders and suicidal behaviour

New Danish research shows that herpesvirus not only causes cold sores but also increases the risk of developing a mental disorder and attempting or dying from suicide. The discovery may be important in understanding how mental disorders develop.

By Kristian Sjøgren

People who have herpes do not just risk getting cold sores when they are stressed or have a cold. New Danish research using data from the Danish Blood Donor Study indicates that being infected with herpes simplex type 1 virus increases the risk of developing a mental disorder and of attempting or dying from suicide.

The results show that Danish blood donors who had herpes had a 1.40 times higher risk of attempting or dying from suicide than a matching control group. Similarly, the risk of developing a mental disorder later in life was 1.44 times higher among blood donors who had herpes at the time they donated blood.

"The unique aspect of our study is that we can definitely conclude that these people were infected with herpes before they developed a mental disorder. It is often unknown whether people were infected before or after being diagnosed with a mental disorder, but we could determine this in our study, in which we discovered an association between having herpes and having an increased risk of developing a mental disorder later in life," explains a researcher behind the study, Janna Nissen, Postdoctoral Fellow, Biobank Unit, Department of Clinical Immunology, Rigshospitalet, Copenhagen.

The research results were recently published in *Psychoneuroendocrinology*.

82,000 Danes studied

The researchers used data from the Danish Blood Donor Study and matched them with data from other patient registries to identify the blood donors who either had or later developed a mental disorder, attempted suicide or died from it.

The Danish Blood Donor Study contains blood samples from 81,912 residents of Denmark, and in collaboration with researchers from the Stanley Division of Developmental Virology, Johns Hopkins University School of Medicine, Baltimore, MD, USA, the Danish researchers analysed the blood samples for herpesvirus and other markers of infection.

The researchers linked this with records in patient registries and found 1504 people with a diagnosed mental disorder and 353 people who had attempted or died from suicide.

The study is the first suggesting that herpes may be associated with attempting or dying from suicide.

Herpes probably not only erupts as cold sores around the mouth

"Being able to examine the time perspective here and determine that the risk of developing a mental disorder increases after a person has been infected with herpes is very interesting. The fact that we can see that they were infected before the risk of mental disorder increased is unique and indicates an association," says Janna Nissen.

Herpes infects the brain

According to Janna Nissen, the association between herpes, mental disorder and the risk of suicidal behaviour is presumably related to how the herpesvirus infects the body.

People infected with herpes have the virus permanently; it erupts when the immune system is suppressed, such as when people experience stress, when many people with herpes get cold sores.

Herpes probably not only erupts as cold sores around the mouth but also, among some people, as "cold sores" in the brain. These can potentially affect the state of mind of the person with herpes.

"This happens with other types of infection, such as people who develop toxoplasmosis caused by Toxoplasma gondii, which can also affect their mental state. Some people's immune system probably keeps herpes under control, and then it never manifests as anything but an occasional cold sore, whereas other people may be quite affected mentally because their immune system does not suppress the infection," explains Janna Nissen.

In general, strong scientific evidence indicates that some chronic infections are associated with various types of mental disorders.

Can treating herpes more rapidly avoid mental disorders?

These new results are the first step towards new understanding of how chronic infections by common viruses can strongly affect the people who are infected.

Janna Nissen imagines that future research confirming this will provide an incentive to more closely assess the treatment of people with herpes, who may need to be treated much earlier and more intensively than they are today.

In addition, the study may also be useful to make researchers aware of the molecular mechanisms underlying the development of mental disorders.

"We may conclude that it could be worthwhile to try to treat people with herpes early before the virus damages the brain, potentially resulting in a mental disorder," says Janna Nissen.

Determining the role of the immune system in developing mental disorders

The future work of the research group will examine the interaction between humans, viruses, the brain and mental disorders.

The researchers would like to investigate various biomarkers among people with herpes. They would aim to find the molecular causes for why some people with herpes have an increased risk of developing a mental disorder and others do not.

The Danish Blood Donor Study has stored blood samples from all previous blood donations in Denmark, and thus the researchers can go back in time and examine the blood for various biomarkers.

"Maybe it all depends on the immune system, which suppresses the virus for some people but not for others. We hope that our discoveries can be part of understanding the mechanisms of mental disorders," says Janna Nissen.

"Herpes simplex virus type 1 infection is associated with suicidal behavior and first registered psychiatric diagnosis in a healthy population" has been published in Psychoneuroendocrinology. Several authors are employed at the Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

The brain's fear centre also controls hunger

The amygdala is the area of our brain that plays a key role in processing emotions, including fear. New research shows that it also regulates our appetite through interaction between a vital signalling substance in the immune system and one of the most important metabolic hormones. Mice without this immune substance become obese. In addition to contributing to knowledge about the interaction between the brain and the gut, this new knowledge is especially important in treating people with autoimmune diseases.

By Morten Busch

Few people question the role of the brain in making us feel hungry. Nevertheless, recent research has focused on the opposite: namely, whether signals from our intestinal system may control hunger. Interaction between brain signals, gut hormones and especially the gut bacteria may be key to understanding the worldwide obesity epidemic. Researchers have now revealed that an intestinal hormone, interacting with a signalling substance in the immune system, affects the tiny fear centre in the brain called the amygdala and that this interaction can influence whether we become overweight or not.

"Mice that lack interleukin-6 (IL-6) become obese, but if you inject it into their brain, they return to their normal weight again. Our experiments show that the amygdala significantly regulates this process through interaction between glucagon-like peptide-1 (GLP-1) and IL-6, a key signalling substance in the immune system. This is especially important knowledge in treating people with autoimmune diseases to avoid excessively suppressing the IL-6 signal so that the people become overweight," explains John-Olov Jansson, Professor, Department of Physiology, Institute of Neuroscience and Physiology, University of Gothenburg, Sweden.

Close interaction

This discovery can be traced back to 2002, when the Swedish researchers published a remarkable study in Nature Medicine. While investigating what happens to mice lacking cytokines, essential signalling substances in the immune system, they noticed that mice lacking IL-6 became obese.

"When we injected IL-6 into the mice lacking the protein, nothing happened immediately – until we injected it into the brain. This helped the mice to return to their normal weight. Since then, we have tried to understand why IL-6 is associated with obesity if it is secreted from fat tissue into the blood, whereas injecting it into the brain inside the blood–brain barrier helps to prevent obesity. Our new results show that the amygdala is responsible for some of this effect."

The facts that the amygdala regulates hunger and that GLP-1 also plays a role are well known. The researchers therefore wanted to determine whether GLP-1 and IL-6 interact, and the results showed that they interact very closely.

"These proteins bind to the same neurons, so they obviously influence each other. We do not yet know exactly how, but IL-6 seems to be unable to cross the blood–brain barrier. Conversely, GLP-1 can do this, and GLP-1 seems to induce an IL-6 signal, but it is too early to conclude anything about this complementary signalling. Clearly, however, the interaction between GLP-1 and IL-6 in the amygdala affects appetite and metabolism," says Fredrik Anesten, Department of Physiology, Institute of Neuroscience and Physiology, University of Gothenburg, Sweden.

Autoimmunity and obesity

Although more studies are needed before researchers can elucidate how GLP-1 and IL-6 interact in the amygdala and thus help control our desire for food, the new findings already have obvious uses: treating people with autoimmune diseases.

"Today, people with autoimmune diseases are treated with various types of medicine that suppress their symptoms. However, our study shows that we may need to avoid excessively suppressing the IL-6 signal, since this can strongly affect the metabolism and health of these people," explains Fredrik Anesten.

Using IL-6 as a general remedy against obesity is probably not an option for now. It actually affects the brain very differently from the rest of the body. Thus, although IL-6 in the brain can suppress hunger and lead to weight loss, this is less obvious elsewhere in the body.

"IL-6 is often present in fatty tissue together with other inflammatory signalling substances in the immune system such as tumour necrosis factor-alpha. Together these have a devastating effect on our metabolism, in stark contrast to the effect of IL-6 interaction with GLP-1. We therefore need to understand IL-6 and its interactions better before we can use it for treatment," says John-Olov Jansson.

"Interleukin-6 (IL-6) in the central amygdala is bioactive and co-localized with glucagon-like peptide-1 (GLP-1) receptor" has been published in the Journal of Neuroendocrinology. In 2013, the Novo Nordisk Foundation awarded a grant to co-author Karolina P. Skibicka for the project Can GLP-1 Modify Reward Behaviour and Circuitry to Reduce Reward Cravings? Answers from Rodents and Men in Health and Disease.

Hormones and metabolism

Content

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Childhood obesity associated with higher risk of colon cancer in adults

A Danish study has shown that adults who were overweight as children have an increased risk of developing colon cancer, but not rectal cancer. However, losing weight can prevent this. Similarly, overweight young adults have a lower risk of developing colon cancer if they were not overweight in childhood. A researcher says that early intervention on obesity can also save society money.

By Kristian Sjøgren

Researchers have long known that obesity in adults is linked with an increased risk of developing various types of cancer, including colon cancer. A Danish study shows that this risk also applies to childhood obesity, and not just to adults. People who were overweight as children have a greater risk of developing colon cancer in adulthood. Childhood obesity is also correlated with an increased risk of developing colon cancer: the higher the body mass index (BMI), the greater the risk. The study also shows that boys and girls do not differ in how overweight increases risk of developing colon cancer.

"This is not a dramatic increase in the risk of developing colon cancer, and people do not definitely develop colon cancer just because they were overweight as children. However, we found a statistically significant increased risk of about 20%," explains a researcher behind the study, Thorkild I.A. Sørensen, Professor, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

The study has been published in the European *Journal of Epidemiology*.

Study includes 260,000 Danish children

In the study, the researchers compared BMI data from school records for children born in 1930–1972 with the incidence of colon and rectal cancer from the Danish patient registries. The study included 260,000 Danes, of whom 2676 developed colon cancer and 1681 rectal cancer as adults.

When the researchers compared the study group's data on BMI as children with the risk of developing these two types of cancer, they discovered that the adults who were overweight as children had a greater risk of developing colon cancer, but not rectal cancer. The increase in risk was about 20%, and this should be compared with the fact that about 1 in 1000 adults in the study group developed colon cancer.

"Researchers have long known that obesity in adults increases the risk of colon cancer, but the new aspect of this study is that childhood obesity also increases the risk," says Thorkild I.A. Sørensen.

Unknown reasons for link between obesity and colon cancer

Researchers do not know how obesity is causally linked to the development of colon cancer, but Thorkild I.A. Sørensen speculates that obesity results in metabolic changes that may affect cells in the colon. This may involve hormonal changes that increase the risk of cancer-promoting mutations. More specifically, these hormonal changes may affect the mucosal barrier of the colon. This theory could also explain the differences in the risk of developing colon versus rectal cancer.

"The colon and rectum differ greatly. The contents of the colon may play a role and could interact and result in the development of cancer. This does not apply to rectal cancer, because the rectum normally only contacts the contents of the colon during defecation," explains Thorkild I.A. Sørensen.

Long-term obesity increases the risk of colon cancer

To follow up the study in the European Journal of Epidemiology, the researchers conducted another study examining how changes in BMI throughout childhood and into adulthood are associated with the risk of developing colon cancer. This study has been published in the International Journal of Obesity.

The researchers used the data from school records for the birth years 1930–1972 and data from the medical examinations conducted by medical conscription boards among men in young adulthood. This method enabled them to determine the BMI of 65,000 men at three points in their young lives: 7, 13 and about 20 years.

Of these 65,000 men, 751 developed colon cancer as adults. The researchers found that the men who had been were overweight at age 7, 13 and 20 years had almost three times higher risk of developing colon cancer than the men who had not been overweight at any of the three times. Conversely, the increased risk almost completely disappeared if the men had either lost weight from the age of 13 to 20 years or if they had been overweight at 20 years old, but not as children.

"The important message is that long-term obesity seems to be the decisive factor in increasing the risk of colon cancer. The risk disappears if overweight is eliminated. Further, a person who was not overweight as a child but becomes overweight later in life may not have a similarly increased risk," says Thorkild I.A. Sørensen.

Results should lead to interventions on childhood obesity

Thorkild I.A. Sørensen emphasizes four aspects of the new research results.

- He hopes that the results will inspire other researchers to examine this topic in greater depth and to help discover why the development of colon cancer is associated with overweight and obesity.
- The study indicates a topic that should be examined further: something may happen as children become overweight that increases the risk of developing various types of cancer in adulthood if the overweight is not eliminated in time.
- The research definitely demonstrates that being overweight has major health consequences for children. This should therefore be on the health policy agenda.
- Finally, cancer is an economic burden for society, and early interventions on childhood obesity may therefore bring economic benefits in the long term – also because many other costly conditions are associated with obesity, such as heart disease and diabetes.

"It may potentially be far better for the population and much less expensive to treat children for obesity – or, even better, prevent them from becoming overweight – than treating people for the many diseases arising later in life. Early effective interventions would clearly be better," concludes Thorkild I.A. Sørensen.

"Childhood body mass index and height in relation to site-specific risks of colorectal cancers in adult life" was published in European Journal of Epidemiology. Thorkild I.A. Sørensen, a co-author of the article, is affiliated with the Novo Nordisk Foundation Center for Basic Metabolic Research.

Research reveals how exercise keeps us healthy

Exercise and muscle training have many benefits. They prevent cancer and cardiovascular disease, and they stabilize blood glucose levels among people with type 2 diabetes. This is one reason why researchers have been trying for decades to understand how physical activity confers so many benefits. New research reveals the molecular mechanisms involved, and the researchers hope that people who have difficulty exercising can be stimulated artificially so that they can still enjoy the many useful health benefits.

By Morten Busch

”Learning to understand why and especially to simulate how physical activity helps people with diabetes, cardiovascular disease or cancer requires understanding the tiny details.”

Thomas E. Jensen

A rising pulse, beads of sweat and more rapid breathing. Although exercise is tough, we know it is good for us, but we are not sure why. For decades, researchers have tried to understand the molecular mechanisms that trigger major metabolic responses in muscles that help to optimize athletic performance and to prevent and treat ageing- and lifestyle-related diseases, such as type 2 diabetes.

“Unlike healthy people, people with type 2 diabetes cannot rely on insulin getting the muscles to take up glucose. If they exercise, however, the glucose is transported through glucose transporter type 4 (GLUT-4). However, we are only now beginning to understand through our new experiments how muscle contractions stimulate that transport. Now we hope to find a way to artificially stimulate these same mechanisms so that we can help people who are not physically able to exercise,” explains Thomas E. Jensen, research group leader and Associate Professor, Department of Nutrition, Exercise and Sports, University of Copenhagen.

Crucial link to health effects

Researchers have been on a long and complicated search for the mechanisms that give exercise the same stabilizing effect on blood glucose as insulin among people with type 2 diabetes. For more than 60 years, researchers have been trying to understand how glucose is transported into the muscles, since this is a key factor in keeping blood glucose levels low. Insulin helps this process among people without diabetes. However, for people with diabetes, either insulin does not work optimally (insulin resistance) or the body does not produce enough insulin, and the muscles require other mechanisms.

“Our goal was therefore to try to understand the insulin-dependent transport of glucose through GLUT-4: how does an intact organism using muscles during physical activity stimulate this transport? Laboratory experiments on muscle tissue grown outside the body have shown that reactive oxygen species, including free radicals, are important signals for increasing transport. However, investigating whether this is also the case when muscles are functioning normally inside the body and the origin of these reactive oxygen species has been difficult technically.”

The researchers therefore adopted new techniques to investigate these mechanisms in mice and humans. By combining advanced microscopy, genetic biosensors, genetically modified mouse models and muscle biopsies from people, they examined for the first time the relationship between glucose uptake in muscles and the various probable sources for producing reactive oxygen species during exercise corresponding to a brisk 20- to 30-minute run.

“We especially focused on the NADPH oxidase 2 (NOX2) enzyme, which produces the reactive oxygen species. Exercise increased the production of reactive oxygen species through NOX2 in mice and among people. However, when we removed NOX2 in mice, the mice also lost the positive effects of exercise on glucose uptake. So, during moderate physical activity, the NOX2 enzyme is the primary source of production of the reactive oxygen species that are necessary for transporting glucose into the muscle cells.”

Reactive oxygen species must be tamed

The researchers managed to determine the precise molecular details of the beneficial effects of exercise on stabilizing blood glucose in even greater detail. For example, they discovered that the NOX2 enzyme works only in the presence of two other proteins, Rac1 and p47phox, and this knowledge may prove to be the key to making the new research useful.

“Learning to understand why and especially to simulate how physical activity helps people with diabetes, cardiovascular disease or cancer requires understanding the tiny details. They will be key in finding the targets for future medicines so that we can help the people who cannot exercise to get its beneficial effects.”

However, if the targets for future medicines have too central a role in the body, the medicines may have not only positive effects but also other negative side-effects. For example, researchers already know that the production of the same reactive oxygen species that benefit adapting to physical activity is chronically elevated among people who have several specific diseases. These reactive oxygen species can damage cells and even destroy them if they are not tamed in the body.

“Hormesis is a concept in which a minor stress effect, here in the form of increasing reactive oxygen species during exercise, triggers several adaptations that improve health. Conversely, the excessive production of reactive oxygen species – oxidative stress – contributes to several diseases and perhaps even ageing, which explains why people take antioxidants that eliminate reactive oxygen species. Surprisingly, regular exercise also strengthens many of the body’s own antioxidant mechanisms, providing resistance to oxidative stress. The new breakthrough is that we can now distinguish between the different sources of reactive oxygen species in muscle cells inside the body instead of just examining the whole cell. More detailed understanding is an important step towards fundamentally understanding how reactive oxygen species contribute to health and disease. In the long term, this may enable the development of drugs that stimulate some of the positive effects of exercise.”

“Cytosolic ROS production by NADPH oxidase 2 regulates muscle glucose uptake during exercise” has been published in Nature Communication. In 2015, the Novo Nordisk Foundation awarded a grant to Thomas E. Jensen for the project Understanding Skeletal Muscle Plasticity in Health and Disease. In 2019, the Independent Research Fund Denmark and Danish Diabetes Academy awarded grants to enable him to continue his research.

Pregnant women becoming increasingly overweight

New Danish research shows that the body mass index (BMI) of pregnant women is increasing over the years. Their newborn babies are also becoming larger, which increases the child's risk of becoming overweight. A researcher is concerned.

By Kristian Sjøgren

Pregnant women in Denmark are becoming increasingly overweight over the years. The percentage of pregnant women in Denmark with a prepregnancy BMI (weight in kg/(height in metres)2) exceeding 25, which is the threshold for overweight, rose to 34% in 2012. The percentage of pregnant women who were obese (BMI > 30) before pregnancy reached 13% in 2012.

Women who are overweight during pregnancy often give birth to overweight babies. These children have an increased risk of becoming obese throughout their lives, in a vicious circle that currently seems endless.

Overweight women also have a greater risk of experiencing all kinds of problems in pregnancy, including pre-eclampsia, and they frequently have problems during childbirth requiring caesarean section, vacuum extraction or forceps.

"Since we know that being overweight during pregnancy is associated with so many problems, it is alarming to see that pregnant women in Denmark are becoming increasingly overweight as the years go by. Unfortunately, this does not just apply to pregnant women but permeates society as a whole," explains Per Ovesen, Consultant Obstetrician, Aarhus University Hospital and Professor, Department of Clinical Medicine and Department of Obstetrics and Gynaecology, Aarhus University.

The new study was published in *Acta Obstetrica Gynecologica Scandinavica*.

Overweight pregnant mothers have overweight babies

The study examined a cohort from the Danish Medical Birth Registry including all women in Denmark who gave birth in 2004–2012. During this period, the Registry recorded data from 572,321 births, corresponding to 99.8% of all births in Denmark. The percentage of pregnant women who were overweight increased from 31.9% in 2004 to 34.2% in 2012, and the percentage who were obese increased from 11.0% to 12.8%.

"These are tremendous increases, but they reflect the general trend in society, which unfortunately is that more and more of us are becoming overweight," explains Per Ovesen.

The problem with pregnant women being overweight is that there are no benefits. When a mother is overweight and overeats during pregnancy, the baby also gets bigger, but the birth canal through which the child has to pass during delivery does not get bigger. Some newborns simply end up being too big to be born naturally.

"Unfortunately, we have created a trend in which heavier mothers give birth to heavier children, who then become heavier mothers, who in turn give birth to even heavier children. This is not good," says Per Ovesen.

Gaining more weight for each pregnancy

Per Ovesen's research also shows that giving birth to more children exacerbates the problems. Women naturally gain weight during pregnancy, but unfortunately few revert to their original weight. The more children they have, the more overweight they become.

"In extreme cases, women gain 20 kg per pregnancy but lose only 10 kg afterwards. This is not good. Women need to revert to their previous weight after giving birth. If women give birth to three children, over the three pregnancies they could end up gaining 30 kg, and that is unhealthy," says Per Ovesen.

Multiple births increase the risk of type 2 diabetes

Connecting these new research results with other research from Per Ovesen's group makes the problems even more obvious.

Another study from the research group has shown that giving birth more times increases the risk of developing type 2 diabetes. It simply happens because women gain weight during each pregnancy without losing it afterwards, and obesity is inextricably linked to the risk of developing various metabolic disorders.

The same risk applies to the next generation.

Women usually become insulin resistant during pregnancy. This corresponds to a mild form of diabetes.

The placenta ensures that pregnant women become insulin resistant, so that they do not absorb sugar from the food they eat, diverting it to the fetus instead.

This evolutionary mechanism made very good sense 10,000 years ago when food was often scarce and infant mortality was high. Today, the mechanism just produces heavier babies because many pregnant women eat more than they and their unborn baby actually need.

"It is pure evolution theory, and it worked very well then, but now it is really bad," says Per Ovesen.

Breastfeeding to lose the weight

The worldwide trend is that people are becoming increasingly overweight, and although Denmark has a major problem, many other countries have even greater problems. The United States and Mexico are perhaps the most obvious examples of countries that are really struggling with runaway obesity epidemics.

According to Per Ovesen, reversing the epidemic requires measures that can break the vicious circle of overweight mothers giving birth to overweight children.

Women need to be informed that they should revert to their original weight after giving birth and should not accept being 5 or 8 kg heavier. The next time they get pregnant, they might gain even more weight, and eventually their children will pay the price by passing on the mother's legacy to their children in the form of overweight.

"The best way for women to tackle this is to breastfeed. Breastfeeding women burn large amounts of energy, and they produce milk that also depletes energy and diverts it into their newborn baby. In addition, women should not just sit on the couch and eat unhealthy food but instead get out and take long walks so that they can get their bodies back in shape and ready to get pregnant again," explains Per Ovesen.

Fortunately, Per Ovesen's research has shown that pregnant women who get the right information are rapidly motivated to ensure the health of their unborn child, making it easier for them to be healthy during pregnancy.

Unfortunately, this house of cards may collapse when women have to take care of themselves and shed the excess weight after giving birth. Many still struggle with that.

"Associations between parity and maternal BMI in a population-based cohort study" has been published in Acta Obstetrica Gynecologica Scandinavica. The Novo Nordisk Foundation awarded Per Glud Ovesen, Professor, Department of Clinical Medicine and Department of Obstetrics and Gynaecology, Aarhus University, a grant in 2015 for the project Overweight, Diabetes and Pregnancy: the Effects on Birth and Pregnancy and on the Risk of the Mother and Child of Developing Obesity and Type 2 Diabetes Later in Life.

You can’t score if you don’t shoot

Three decades ago, only a small number of researchers talked about gut hormones. Today, many people view these hormones as building blocks for developing new therapies to confront the obesity and diabetes epidemics that are sweeping the world. Daniel J. Drucker has studied the molecular mechanisms and physiological functions of hormones for 35 years. His studies unravelling their biological actions have led to several discoveries and the development of life-changing therapies. For his outstanding contributions, he is receiving the 2019 EASD–Novo Nordisk Foundation Diabetes Prize for Excellence.

By Morten Busch

Everyone knows about insulin – at least by name. However, fewer people nod knowingly if you mention incretins. Although the incretin concept was established as early as 1932, incretin hormones remained obscure for more than 50 years. Daniel J. Drucker was one of the pioneers in the mid-1980s who rekindled interest in the action of incretins, principally glucagon-like peptide-1 (GLP-1). Today, the two major incretins, GLP-1 and gastric inhibitory polypeptide (GIP), have become crucial in developing new therapies for diabetes, cardiovascular diseases and obesity.

“At that time, only a few researchers, such as Joel Habener, Jens Juul Holst, Stephen Bloom and I, were interested in incretins and related gut hormones. Although I was convinced that they were important, I could not have imagined that this knowledge would be translated into multiple new therapies that help people with severe metabolic disorders today. But that’s how science is. If you do enough experiments, and the science is independently validated by peers, sometimes you build a story over time and ultimately hit the translational jackpot. That scenario can be one of the most exciting rewards arising from research in the medical sciences,” says Daniel J. Drucker, Professor of Medicine, University of Toronto and Senior Scientist, Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada.

Today, Drucker is widely recognized for his research in the physiology and pharmacology of GLP-1 and GLP-2 – and especially his success in translating the basic science into therapies for people with obesity and diabetes by helping to develop dipeptidyl peptidase-4 (DPP-4) inhibitors and GLP-1 receptor agonists. This work is the basis for Daniel J. Drucker receiving the 2019 EASD–Novo Nordisk Foundation Diabetes Prize for Excellence.

No room in the field

Daniel J. Drucker’s family believed in working hard to achieve success in life. His parents survived the Holocaust, and this had a lasting impact not only on them but also on Daniel, who has never taken life, health and freedom for granted. His constant motivation does reflect the gift of opportunity his parents provided for him.

“My father, Ernest, was periodically involved in inventions and patents, including solar energy, transport and constructing the modular homes of the future. His entrepreneurial spirit meant growing up in a home in which life could be fantastic, but at other times we had to move from a nice house to a small apartment because he had gone bankrupt again. So I ultimately chose a more stable career in medicine instead of a career in business.”

Daniel J. Drucker graduated in medicine from the University of Toronto in 1980 and received postgraduate training at Johns Hopkins Hospital, the University of Toronto, Massachusetts General Hospital and Harvard Medical School, where, following the advice of his mentors in Toronto, he planned to work on problems related to thyroid hormones. However, when he arrived at Harvard, he was told that there was no room for him to work on the thyroid projects in the lab.

“I was informed that thyroid research was not an option, so if I were to stay there, I had to focus on the recently cloned glucagon gene and its related hormones instead. At that time, I was not interested in that field, but I had no other options and just had to get on with it.”

”We soon became interested in studying how GLP-1 works on our appetite and energy balance and in understanding what happens to GLP-1 action during the development of diabetes and obesity.”

Daniel J. Drucker

A whole arsenal of incretins

Fortunately, a few years earlier a significant breakthrough had been made in the endocrinology of the digestive system. At that time, most of the interest in glucose-lowering hormones focused on insulin, with secondary importance attributed to glucagon, which raises glucose. However, in the early 1980s, when the groups of Joel Habener and Graeme Bell cloned and sequenced the genes for proglucagon, the precursor of glucagon, they got a surprise.

“The gene contained two additional glucagon-like sequences encoding two glucagon-like peptides that subsequently became known as GLP-1 and GLP-2. GLP-1 turned out to increase insulin secretion significantly, so as an endocrinologist, I quickly realized that this nascent field in which I found myself had enormous potential.”

As the researchers looked more closely, they found that the proglucagon gene is expressed in the pancreas, intestines and brain. Although the proglucagon protein produced is identical in all mammalian tissues, the profile of liberated peptides differs depending on the type of tissue. In 1987, after completing postdoctoral training, Daniel J. Drucker was appointed Assistant Professor of Medicine at the University of Toronto.

“We soon became interested in studying how GLP-1 works on our appetite and energy balance and in understanding what happens to GLP-1 action during the development of diabetes and obesity. It also became apparent that incretin receptors are expressed in multiple tissues in which incretin action was not yet clearly defined.”

A poisonous lizard helps

The researchers quickly realized the enormous potential of regulating GLP-1 and related peptide activity. However, using natural GLP-1 in therapy poses a major problem. It is not very stable and is degraded quickly by other enzymes and cleared rapidly in the body.

“This is not a problem for GLP-1 physiology in a healthy body, since GLP-1 is produced continuously. However, it needs to be much more stable for medical therapy, and there was virtually no success in developing GLP-1 therapy for many years despite its enormous potential. So even though we knew it worked, we had begun to doubt that anyone would ever crack the code of successful GLP-1 drug development.”

Help arrived more than a decade later from a somewhat unexpected quarter. At the Veterans Affairs Medical Center in the Bronx, researcher John Eng tried to develop a method for detecting new hormones. During his research, he found an interesting hormone in the venom of the Gila monster, a poisonous lizard that lives in the southwestern United States. When he examined the venom to determine which hormones were present, he got a bit of a surprise. He discovered not only the hormone he was looking for but also a new one: exendin-4. He looked it up in a database and found that it is very similar to the human hormone GLP-1.

“The big difference was that exendin-4 is much more stable, and this was an important

breakthrough in GLP-1 therapy. The pharmaceutical industry later tested and further developed synthetic exendin-4 into the drug known as exenatide. But Eng's belief, passion and thoroughness made the difference for the development of exenatide. Enthusiasts like Eng are needed to achieve key breakthroughs in research, but it is equally important that someone considers the idea and asks whether we can use this for something."

Short-bowel syndrome

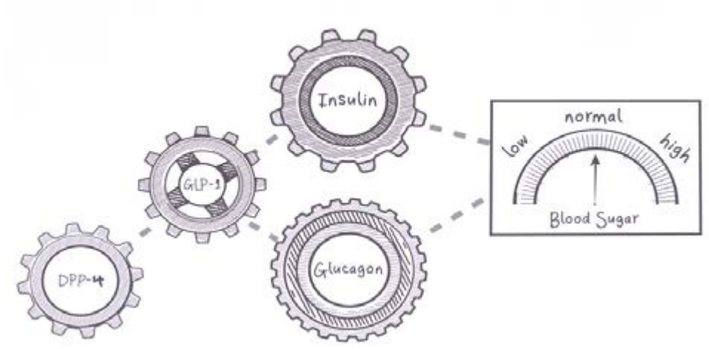
Throughout his career, Daniel J. Drucker has been dedicated to translating his findings into therapies to help people. He credits both his father's interest in patents and his supervisor in Boston, Joel Habener, who both introduced him to the concept of intellectual property.

"While working in Boston, I made a very interesting discovery: GLP-1 stimulates insulin secretion and insulin biosynthesis directly in islet cells. Not long thereafter, suddenly one day when I arrived at the laboratory, my mentor, Joel Habener, had requested my notebook, containing all my experimental plans, results and data. My supervisor had become very interested in my data, and a patent had to be filed before we went further and published the work. This experience taught me that filing a patent after making an important observation in medicine can help lead to the development of new therapies and be critical for attracting the financial backing to develop it."

Daniel J. Drucker made another intriguing observation in 1995 while developing a new cell line for studying glucagon-like peptides. His research has always been extremely thorough, strongly focusing on how the hormones affect various parts of the body. Serendipitously, this cell line work led to the first discovery of the action of GLP-2, a hormone similar to GLP-1.

"We did not really know what GLP-2 does at that time, but we found that it is a strong growth factor that works specifically in the intestine."

With his background in medicine, Drucker knew the symptoms of short-bowel syndrome, in which people's small intestines are so short that they have difficulty in absorbing fluids and food. Many of these people need hours of life-sustaining



intravenous infusions every day and have difficulty in living a normal life, since they cannot eat normally or travel very far away from home.

"We examined the effect of GLP-2, first in animals and later in humans, and found that it can restore enough functional intestine to make a difference. With the help of a local biotechnology company, we managed to develop this into a therapy so that, today, people with short-bowel syndrome only need to inject it once a day. Many of these individuals can markedly reduce or even discontinue the fluid infusions, helping them to live a near-normal life. Magical moments like this enable researchers to live with our many negative or disappointing results in research, but you can't score if you don't shoot and you need to take multiple shots at the goal."

Protecting the heart

Incretins gradually took off, as more stable variants of GLP-1 emerged. The research by Daniel J. Drucker and his colleagues therefore changed from being somewhat exotic to being a centre of attention for pharmaceutical research and clinical investigation. Drucker's research was key to this trend. He showed that GLP-1 stimulates insulin secretion, cloned complementary DNA (cDNAs) for GLP-1 in the gut and brain, cloned the lizard cDNAs encoding exendin-4, developed a cell line for studying GLP-1 production and created mouse lines that did not have GLP-1 receptors.

"What caught my attention early on, however, beyond understanding how GLP-1 controls glucose, was the importance of understanding how it is produced and functions at various sites in the body. There are incredibly large differences in how GLP-1 works in many tissues. Attempting to understand how GLP-1 works in various tissues made us realize how large and complex this field of biology is."

One advantage Daniel J. Drucker had was that he worked at a large academic medical centre with many skilled researchers, including cardiologists. Together with colleagues, his studies showed that GLP-1 proved to have yet another benefit that may turn out to be as important as its effects on diabetes and weight loss.

"We realized that GLP-1 can inhibit the development of experimental heart disease, and we therefore set out to understand the underlying mechanisms. It turned out that activating the receptor for GLP-1 strongly protects the heart."

Drucker conducted comprehensive studies of heart rate, blood pressure, arteriosclerosis, cardiac ischaemia, blood flow and inflammation in the early 2000s that clarified the scientific basis for how incretins protect the heart.

"Although many of the new GLP-1-based medicines were known to be effective in reducing blood glucose or body weight, the positive cardiovascular effect, evident in large human trials, has surprised us all. The medicines reduce the number of heart attacks and strokes and decrease cardiovascular death. GLP-1 therapy seems to address inflammation, diabetes, obesity and cardiovascular complications, providing many reasons to use these GLP-1-based medicines."

Unexpected links between the incretin system and inflammation

In seeking to understand the dynamics of incretins, Daniel J. Drucker also focused in the late 1990s on DPP-4, an enzyme that degrades incretin hormones. DPP-4 inhibitors rapidly gained attention by regulating both insulin and glucagon concentrations in animals and people with type 2 diabetes. DPP-4 turned out to have a very close and interesting connection to the incretins, and Drucker's group, working with DPP-4 knockout mice and DPP-4 inhibitors, immediately saw the potential and tried to understand how the enzyme worked.

"DPP-4 was shown to be a key regulator of GIP, GLP-1 and GLP-2 by cleaving and inactivating these hormones. We and other colleagues quickly identified DPP-4 as a key to controlling the degradation of gut incretin hormones responsible for glucose control. Soon the pharmaceutical industry developed a series of DPP-4 inhibitors for treating people with type 2 diabetes."

Not surprisingly, by inhibiting DPP-4, the researchers could enhance the effect of incretins: for example, potentiating GLP-1 and GIP activity in people with type 2 diabetes. However, DPP-4 has a much more complex biology beyond glucose control and is widely distributed in various types of tissues.

"DPP-4 exercises its biological effects through multiple mechanisms. First, it is localized to cell membranes and both signals inside the cells and functions as a critical molecule for inactivating multiple proteins. However, DPP-4 also exists in a soluble form and has signalling functions at many sites in the body. For example, DPP-4 strongly affects our immune system and can act independently of its classical enzyme activity."

DPP-4 is therefore another key link discovered by researchers related to the increasingly clear connections between the immune system, inflammation in the body and diseases such as obesity, type 2 diabetes and cardiovascular diseases.

"Our most recent studies have shown that DPP-4 inhibitors can upregulate soluble DPP-4 and potentially modify inflammation in many types of tissues. This finding may prove to have many important clinical implications, and we hope to understand the importance of this finding in animals and humans."

Keep the pyramid from turning into sand

Daniel J. Drucker's work with GLP-1 and DPP-4 for diabetes, GLP-1 for obesity and heart disease and GLP-2 and short-bowel syndrome has led to new therapies at a higher success rate than the usual in science.

"I usually describe research as having good days interspersed with bad months, with real magic arising on rare occasions, often many years apart. Then, if we are really lucky, working with many colleagues in universities and companies, collectively we can transform this magic into a new therapy that can help people. Most of the time, researchers do not succeed and I would describe our success as being unbelievably lucky to be in the right place at the right time, allowing us to make unique observations."

Because of the increasing importance attached to understanding the scientific basis underlying these new human therapies, a few years ago Daniel J. Drucker stopped doing what he otherwise likes the most: seeing patients. Instead, he has devoted himself to searching for new even better therapies and understanding the detailed actions of peptide hormones. Today, he is more certain that these hormones play important roles in many places in the body, not only affecting blood glucose regulation but also body weight, the cardiovascular system, the immune system and the central nervous system. The science in this field is widely believed to be fundamental for the development of new therapies for metabolic disorders.

"I am sure that in the years to come, we, together with colleagues, will make more progress towards realizing the enormous potential of peptide therapy. Hopefully, this will bring more good news for people with diabetes, heart disease, liver disease and perhaps even individuals with degenerative disorders of the central nervous system, such as Parkinson's disease and Alzheimer's disease. More powerful medicines will be developed, and these will also be created in long-acting forms, which means that people with diabetes will not have to frequently monitor their blood glucose or regularly adjust the dose of their medicines. They will just need to take the medicine once a week or maybe even once a month, or a few times a year, to achieve easier, more effective and safer therapy."

Despite the enormous potential of modern science, Daniel J. Drucker believes that responsible science is best carried out carefully, not by rushing, exaggerating and publicizing new results before being completely certain of their importance and authenticity. In fact, Drucker believes that part of his success reflects the fact that, throughout his career, he has maintained very strict standards for the quality of his scientific output.

"We have to be very, very careful, to get it right. For me, the most important thing has always been to ensure that the results of my experiments can be easily replicated and to ensure that the conclusions I draw are as correct as possible. The journals always like to publish sensational 'amazing' very novel results, indirectly encouraging some researchers to emphasize aspects of their results that may actually be less robust than they would like them to be. However, I think it is incredibly important to remember that enduring science is like a pyramid. Each new research article needs to be a metaphorical brick on which others build, but if you are not careful with the quality of the bricks, the pyramid can become a sand castle that collapses far too easily."

"The Ascending GLP-1 Road From Clinical Safety to Reduction of Cardiovascular Complications" has been published in Diabetes. Daniel J. Drucker is receiving the 2019 EASD–Novo Nordisk Foundation Diabetes Prize for Excellence accompanied by DKK 6 million (€806,000) for his outstanding contributions that have increased knowledge of diabetes.

Recently mapped protein may help to solve the global obesity epidemic

Membrane protein aquaglyceroporin 10 (AQP10) plays a major role in developing obesity. Researchers from the University of Copenhagen have now mapped the protein's structure and how it functions in controlling fat levels in the body's cells. The next step involves determining how this new knowledge can be used for developing anti-obesity medicines and for combating type 2 diabetes and related diseases.

By Kristian Sjøgren

Researchers at the University of Copenhagen have mapped the structure of a membrane protein. This may not sound very sexy, but it is, because this protein plays a role in how the body metabolizes fat. Thus, this protein influences how much of your Christmas food will end up as flab.

Mapping these proteins means that researchers can start to develop medicine that specifically targets such proteins and prevents the body's fat cells from making us fat.

In the long term, this type of medicine may help to combat the world's galloping obesity epidemic while also slowing the development of diseases related to obesity and lifestyle diseases such as type 2 diabetes, cardiovascular diseases and cancer.

In the long term, this type of medicine may help to combat the world's galloping obesity epidemic

"We now know how this protein looks and functions, so we can use computation tools to design molecules that either activate or inhibit this protein and thereby also its function in how the body metabolizes fat. This is the overall perspective," explains a researcher behind the mapping, Pontus Gourdon, Associate Professor, Department of Biomedical Sciences, University of Copenhagen.

This new study, which focuses on the mapping of the protein, was recently published in *Nature Communications*.

A protein removes broken down fat from the body's cells

This complex new research describes adipocytes: the cells in the body that store fat. To accumulate or dispose of fat, fat cells need to have several proteins that transport the fat components in and out of the cells, including glycerol.

The process of breaking fat down in cells is called lipolysis, and this releases glycerol and other substances that can be transported out of the cells.

Pontus Gourdon and his colleagues Kamil Gotfryd, Julie Winkel Missel and Kaituo Wang have characterized the structure and function of the aquaglyceroporin 10 (AQP10) protein, which transports glycerol out of the cells.

More complete picture of exercise and fat metabolism

In addition to the structure of AQP10, the new research also shows how the pH of the fluid inside the cells determines how much glycerol AQP10 can transport.

The more acidic the intracellular fluid is, the more rapidly glycerol can be transported out of the cell. Conversely, neutral or basic fluid slows the whole process.

The link between pH and fat metabolism is actually not new. Research had previously shown that exercise makes the fluid inside the fat cells more acidic, which is directly linked to the breakdown of fat.

The new research consolidates this by showing that the activation of AQP10, required for releasing glycerol, is associated with pH.

"Previously, the structure of AQP10 was little understood, as was how the pH of the intracellular fluid affects its function. We know more now, and this makes sense physiologically," says Pontus Gourdon.

Mapping the structure of a membrane protein is complex

Determining the structure of a protein may sound simple, but it is definitely not. This can best be illustrated by the fact that the researchers from the University of Copenhagen spent more than 3 years on this project, assisted by expertise from many national and international collaborators.

- First, the researchers needed to get yeast cells to produce the protein in sufficient quantities to be able to study its structure.
- Then they had to purify the protein from the cells to a high degree, which is necessary for crystallizing the protein.
- Once the protein was in crystal form, the researchers could generate a model of the atoms in the protein (a structure) using powerful X-rays.
- Then the researchers carried out numerous experiments to characterize the function of AQP10. One type of experiment enabled them to determine how the protein became more active and could thereby transport glycerol more rapidly in more acidic intracellular fluid.
- Finally, the researchers asked collaborating partners in Italy and Portugal to carry out various cell experiments to confirm that the researchers from the University of Copenhagen had found the right mechanism.

"Determining the structure of a protein is no trivial matter. To our knowledge, we actually determined the structure of a human membrane protein for the first time in Denmark. From this perspective, it is unique," explains Pontus Gourdon.

Developing molecules that can become medicine

Now that the researchers understand the structure and function of AQP10, the next step is to develop molecules that can interact with this protein.

Pontus Gourdon and his colleagues and partners in Portugal want to develop and study molecules that modulate the function of the protein such that the transport of glycerol is affected. If the researchers can develop molecules that can accelerate the transport of glycerol, mimicking acidic pH, this will have great pharmaceutical potential.

"This protein may have an important role in metabolizing fat in the body, and molecules that either stimulate or inhibit the function of AQP10 therefore influence the development of obesity and related diseases. This naturally has long-term perspectives, but we have already begun to study specific molecules that will be interesting to examine in this context," say Pontus Gourdon.

Pontus Gourdon explains that, since they now know the protein's structure and function, they will use computational analysis to reveal how they can manipulate the protein's functions using one or several other molecules: structure-based drug design.

Once the researchers have discovered relevant molecules, they will test them in the laboratory to examine how they affect AQP10, cells and the body.

"Human adipose glycerol flux is regulated by a pH gate in AQP10" has been published in Nature Communications. In 2016, the Novo Nordisk Foundation awarded a grant to Pontus Gourdon, a main author, for the project Unravelling Glucose Transceptors Involved in Regulation of Food Intake and Secretion of Incretins and Insulin.

Researchers can now test blood to measure how healthy organs are

Blood has many biomarkers that can reveal the health of most organs in the body. Now Danish researchers have developed a new method to interpret the messages of these microscopic biomarkers. Among other things, researchers can see how weight-loss surgery changes the health of blood vessels and the immune response.

By Kristian Sjøgren

Obesity is associated with the risk of developing arteriosclerosis, non-alcoholic fatty liver disease, cardiovascular diseases, insulin resistance and many other diseases, all of which greatly strain many major organs.

Now Danish researchers have developed a method using a simple blood test that may indicate how healthy the body's organs are.

The discovery means that, in the future, doctors can test a person's blood after, for example, weight-loss surgery thereby enabling them to clinically assess whether the person's organs are getting healthier as the weight is lost.

"We can determine whether the person's health inside the body changes after this surgery or whether it only affects a person's weight," explains Aase Handberg, Clinical Professor, Aalborg University Hospital, a researcher behind the new study.

The research was published recently in *Nutrition & Metabolism*.

Tiny messengers communicate between organs
Microvesicles are tiny messengers that communicate between cells.

Basically, microvesicles are tiny bulges (vesicles) on the body's cells that are shed as small pellets into the blood and other bodily fluids.

Microvesicles are then transported around the body with the blood, bringing many types of molecules from organ to organ. These molecules can include cellular surface proteins or tiny fragments of RNA that can regulate the activity of genes.

In this sense, the microvesicles communicate between parts of the body. For example, microvesicles can be secreted from fat cells and then bind to cells in the liver. The microvesicles thus provide the liver with information about the fat cells.

If the microvesicles contain tiny fragments of regulating RNA, the RNA may also influence the liver cells and thereby the liver's function.

"Microvesicles comprise a fantastic communication system within the body. We also know that microvesicles exist in all bodily fluids, including blood, tears, sweat and urine," says Aase Handberg.

Using microvesicles to see inside the body
In this new research project, Aase Handberg and her colleagues discovered how they could decode the microvesicles and use them as biomarkers. They developed a method for using a simple blood sample to directly detect microvesicles from various organs and thus theoretically use them to determine the health of the liver, blood vessels, immune system, kidneys or pancreas.

If a person has developed non-alcoholic fatty liver disease, the unhealthy accumulation of fat in liver cells, circulating microvesicles reflect this, according to Aase Handberg.

The reason why microvesicles can be used as tiny biomarkers is because these are minute fragments of the organs themselves. They are like tiny microbiopsies of individual cells.

"By testing blood, we can theoretically say something about all the body's organs. So far we have been able to do this for the liver, skeletal muscles, immune cells and the lining of blood vessels. This has major perspectives, especially for the liver, because many diseases are associated with the liver," says Aase Handberg.

A membrane protein associated with the risk of cardiovascular disease, obesity and arteriosclerosis
This study is the latest development in Aase Handberg's research, which began 10 years ago with a membrane protein called CD36.

CD36 is on the surface of cells and imports fatty acids into the cells. It thus plays a role in developing obesity, arteriosclerosis, cardiovascular diseases and other diseases because increasing the uptake of fat in the cells means more fat in the organs.

Many years ago, Aase Handberg showed that the concentration of CD36 in the blood can be measured and that a higher concentration means an increased risk of type 2 diabetes and other complications of obesity.

"Basically, we hypothesize that when a person consumes excess calories, the body has to deposit them somewhere. The body can deposit the extra calories in fat tissue on hips and thighs, but these have limited capacity, so if excess calorie intake continues, the body then begins to deposit the fat in the organs. This is where CD36 has a role," says Aase Handberg.

Antibodies identify specific microvesicles
Aase Handberg has developed a method to identify monocyte microvesicles and endothelial microvesicles in a blood test by using specific antibodies that recognize these types of cells.

Monocytes are the cells that become macrophages, the scavengers of the immune response, and endothelial cells are the cells lining the blood vessels. Both types of cells are very active in developing arteriosclerosis and metabolic syndrome, a precursor of type 2 diabetes.

By using antibodies against CD36, the researchers then discovered how many CD36 proteins cells have on their surface.

The concentration of CD36 in the microvesicles enabled the researchers to determine the extent to which the body deposits fat and activates monocytes and endothelial cells, and this can be directly associated with the health of the body in relation to obesity.

"The wider perspective is to use microvesicles as biopsies because tissue cannot be sampled from many parts of the body. In this study, we primarily examined CD36, but we could also have investigated glucose-transporting molecules, which tell us about the efficiency of glucose metabolism or a membrane protein that is a biomarker for a disease," explains Aase Handberg.

Weight-loss surgery is also good for the organs
The researchers tested the blood of severely obese people before and after gastric bypass surgery.

When the researchers examined the blood tests, they discovered that trial subjects who had undergone the surgery also had a generally reduced concentration of CD36 in the microvesicles from monocytes and endothelial cells.

According to Aase Handberg, this indicates that this surgery not only affects weight but also positively influences the underlying mechanisms that damage the health of severely obese people. These included the accumulation of fat in the organs and general inflammation in the body's immune cells and endothelial cells, which promotes arteriosclerosis.

"The microvesicles show that things are improving. Thus, analysing microvesicles also enables us to determine whether something is wrong or whether to focus on specific health-related areas after weight-loss surgery. For example, if the concentration of CD36 in the liver or in the monocytes or endothelial cells does not decline, then these areas should be in focus in relation to diet and medication in the future," says Aase Handberg.

"Bariatric surgery reduces CD36-bearing microvesicles of endothelial and monocyte origin" has been published in Nutrition and Metabolism. Researchers from the Novo Nordisk Foundation Center for Basic Metabolic Research have participated in the project.

Diet and lifestyle

Content

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Why eating whole-grain products is so healthy

New Danish research shows how healthy eating whole grains is. Compared with refined grains, whole grains lower the risk of cardiovascular disease and diabetes and cause people to lose weight. The probable reason is that, when people eat whole grains, they feel full and therefore eat less because they fill their stomach with dietary fibre. In addition, intestinal bacteria can convert whole-grain products into substances that reduce low-grade inflammation.

By Kristian Sjøgren

Whole grains are healthy! Okay, this is not a revelation, because researchers have known this for a long time. Nevertheless, a new Danish study has delved a little deeper into understanding why whole grains are really as healthy as they are.

The research results show, among other things, that whole grains reduce low-grade inflammation in the body and thus reduce the risk of cardiovascular diseases and type 2 diabetes. The probable reason is that intestinal bacteria love whole grains and convert them into substances that can reduce inflammation. Inflammation is known to increase the risk of all sorts of diseases, from diabetes to stroke. The study also showed that whole grains can help people to lose weight.

One type of whole grain seems especially to be a very good food to add to your meal planning if you want to be healthier.

"Rye very positively affects health and seems to be able to counteract inflammation. The conclusion to our study must be that if you are overweight and at risk of developing cardiovascular diseases or diabetes, you should eat some rye sandwiches," says a researcher behind the new study, Tine Rask Licht, Professor, National Food Institute, Technical University of Denmark.

The new research results were recently published in *Gut*.

Human trials are difficult
The researchers more precisely examined how a whole-grain diet affected 60 overweight Danes.

This was a crossover study: the researchers put the trial participants on either a refined-grain diet or a whole-grain diet for their daily intake of bread, pasta, crackers and other grain-containing foods. The participants ate one of the two diets for 8 weeks, then had no specific diet for 6 weeks followed by 8 weeks with the alternative diet to what they had eaten in the first 8 weeks. So if they initially had eaten whole grains, they ate refined grains during the second 8-week period and vice versa.

The trial participants were basically healthy although overweight.

"The first measurable result of eating a diet with lots of whole grains was weight loss. Compared with a refined-grain diet, the test participants lost about 1 kg in 8 weeks on the whole-grain diet," says Tine Rask Licht.

Whole grains fill you up
Tine Rask Licht attributes the weight loss on whole grains to a reduction in the measured overall energy intake, which is likely to result from an increased sense of being full (satiety) compared with refined grains.

The researchers had matched the whole-grain products with the refined-grain products so they contained about the same number of calories.

The participants ate food other than grains, such as meat and vegetables, but the researchers still found that the people who ate whole grains lost weight.

"They lose weight because they eat less. They eat fewer total calories when they eat whole grains, probably because the whole-grain products fill them up more than the refined-grain products," explains Tine Rask Licht.

Whole grains reduce the risk of cardiovascular diseases and diabetes
However, people losing weight by eating whole grains is not the study's eureka moment. It is that eating whole grains can reduce low-grade inflammation, which is known to affect the risk of developing cardiovascular diseases and diabetes.

The researchers did not investigate the actual development of cardiovascular diseases but instead measured markers of low-grade inflammation in the blood.

Such inflammation is associated with an increased risk of developing cardiovascular diseases and type 2 diabetes, and researchers can use blood tests to analyse the concentrations of various substances (markers) that indicate the body's state of inflammation. The concentrations of these markers in the blood indicate the risk of developing the diseases.

The results show that a diet rich in whole grains can significantly reduce the concentrations of markers of low-grade inflammation in the blood and thus also the risk of developing cardiovascular diseases.

"The quantity of whole grains consumed is directly correlated with the decrease in the concentrations of markers of inflammation."

Tine Rask Licht

"The quantity of whole grains consumed is directly correlated with the decrease in the concentrations of markers of inflammation. This indicates that people at risk of developing cardiovascular diseases or diabetes, for example because of obesity, can benefit from eating lots of whole grains," says Tine Rask Licht.

Rye is healthy
The researchers also examined which types of whole grains had the best effects on markers of inflammation.

In addition to using the blood tests to determine whether the trial participants had eaten whole grains, the researchers could use the signature of the different types of whole grains in the form of various markers to determine whether each participant had eaten predominately wheat, barley, rye or other grains.

The researchers thus compared the anti-inflammatory effect of different types of whole grains and found that consuming whole-grain rye especially benefited markers of inflammation.

"When the blood test showed that a participant ate a lot of rye, we could also see a clear anti-inflammatory effect that helps to protect against a wide range of diseases related to lifestyle. Whole-grain rye is clearly healthy," says Tine Rask Licht.

No change in intestinal bacteria
Another part of the study involved stool samples. The researchers were convinced that eating whole-grain products would change the composition of intestinal bacteria.

Intestinal bacteria affect health in many ways. For example, healthy intestinal bacteria produce several substances that can reduce inflammation. Many of these healthy intestinal bacteria specialize in breaking down the fibre from certain types of whole grains, so the researchers were convinced that a diet with many whole-grain products would change the intestinal bacteria.

However, this did not happen.

"We were surprised that we did not find more change in the composition of intestinal bacteria. However, just because we could not find any effect does not mean it was not there. The bacterial community in the intestines is very complex, and there might have been an effect that was hidden by the individual variation between people. In addition, perhaps our trial participants already ate many whole-grain products, so the effect of the change in diet would affect the intestinal bacteria less strongly than the markers of inflammation and their weight," says Tine Rask Licht.

Tine Rask Licht explains that the intestinal bacteria can nevertheless explain part of the anti-inflammatory effects of whole-grain products. When intestinal bacteria break down whole grains, they transform them into short-chain fatty acids, which are known to be anti-inflammatory.

"We had difficulty in precisely measuring any change in short-chain fatty acids in the blood in our study, but this could be an obvious explanation for the positive effects of whole grains," concludes Tine Rask Licht.

"Whole grain-rich diet reduces body weight and systemic low-grade inflammation without inducing major changes of the gut microbiome: a randomised cross-over trial" has been published in Gut. Researchers from The Novo Nordisk Foundation Center for Basic Metabolic Research participated in the research.

Why drinking coffee may counteract type 2 diabetes and cardiovascular diseases

Danish research has identified substances in coffee that may explain why Denmark's favourite hot beverage can counteract several major noncommunicable diseases. Coffee contains 1500 identified substances, but Danish researchers have discovered one that is especially promising for reducing the risk of obesity, cardiovascular diseases and type 2 diabetes.

By Kristian Sjøgren

Coffee has previously been associated with several positive health effects. According to a population studies, coffee can reduce the risk of cardiovascular diseases, obesity and type 2 diabetes. The inky brew can do this because it reduces the risk of metabolic syndrome, the precursor of type 2 diabetes.

Since coffee affects health so positively, an obvious idea would be to extract one or several of the beneficial substances from coffee and to market them as dietary supplements or medicine. This might help to reduce the accelerating global obesity epidemic.

The problem is that coffee contains 1500 identified substances, and determining which ones are really beneficial has been impossible.

Danish researchers have focused on one that appears promising. In a series of experiments, the researchers examined the effects of various substances in coffee. Although they often left the laboratory with no positive results, they now appear to have hit the jackpot.

"We have discovered a substance in coffee that looks especially promising as a dietary supplement. In a broader perspective, a supplement like this may help to minimize the global burden of obesity and type 2 diabetes," explains a researcher behind the new study, Søren Gregersen, Senior Physician, Department of Clinical Medicine, Aarhus University.

Søren Gregersen's research on the various substances in coffee has been published in such journals as *Journal of Natural Products and Nutrients*.

Coffee makes rats lose 10% of their body weight

Søren Gregersen and colleagues selected various substances from coffee and examined them for slimming and blood glucose–lowering effects.

They fed 24 rats a special diet designed to induce metabolic syndrome. They then divided the rats into three groups and administered 1) coffee, equivalent to 4 cups daily for a person; 2) water; or 3) a sample of the target substances extracted from coffee. This method enabled the researchers to examine the effects of the compounds cafestol, caffeic acid, trigonelline and 5-O-caffeoylquinic acid.

"This was like a fishing expedition aiming to find some of the promising substances in coffee grounds," explains Søren Gregersen.

The researchers obtained their results during a 14-week trial in which the rats consumed coffee, water or the various target substances. First, the study confirmed that coffee slims and lowers blood glucose in rats and thus confirmed population studies in humans. During the 14 weeks, the liver fat content was reduced by half, the fatty acids circulating in the blood declined and body weight fell by 8–10% in the rats consuming coffee. These rats were also more sensitive to insulin, consumed less food and had lower blood glucose levels.

"This confirms earlier studies indicating that coffee possesses these effects. Our trial shows that coffee contains several substances that improve the metabolism of fat and sugar," says Søren Gregersen.

Promising substance for a dietary supplement

The researchers did not find the promising effects for caffeic acid, trigonelline and 5-O-caffeoylquinic acid. The rats consuming these substances metabolized sugar and fat similarly to the rats that only consumed water.

But cafestol seemed promising based on several experiments, because the rats consuming cafestol had almost exactly the same effects on weight loss and fat and glucose metabolism as the control rats consuming coffee.

Cafestol comprises 0.4–0.7% by weight of Arabic coffee (60–70% of the global coffee bean production), and brewed French press coffee has the greatest amounts. Filtering the coffee traps the cafestol in the filter, with little ending up in the brewed coffee.

"Cafestol seemed potent and effective in reducing fat in the liver and blood and controlled blood glucose better. However, we do not yet know whether this is the only substance that has these effects or whether combining several substances produces the beneficial effects of coffee," explains Søren Gregersen.

Trials on people with prediabetes

Søren Gregersen and colleagues have decided to study cafestol further. Their goal is to examine whether cafestol is suitable as a dietary supplement or possibly as medicine that can be deployed in battling obesity and type 2 diabetes. However, this means trials involving people.

The researchers are currently developing a protocol for a clinical trial involving people with prediabetes. If the research on cafestol has discovered something that can mitigate metabolic syndrome, then hopefully this will also influence the development of type 2 diabetes.

At least this is what they hope.

"We need to find money to carry out such a trial, and we also need to investigate how we can sustainably extract cafestol from coffee grounds, for example. The further research has many exciting perspectives, and we are well on the way," concludes Søren Gregersen.

"Effects of Unfiltered Coffee and Bioactive Coffee Compounds on the Development of Metabolic Syndrome Components in a High-Fat-/High-Fructose-Fed Rat Model" has been published in Nutrients. The Danish Diabetes Academy, supported by the Novo Nordisk Foundation, provided funding for this project in the form of a doctoral fellowships to Ph.D. Pedram Shokouh from Department of Clinical Medicine at University of Aarhus.

A father's overweight can influence his children's risk of disease

Epigenetics plays a major role in a child's risk of developing such diseases as obesity, type 2 diabetes, schizophrenia and autism. Danish research suggests that, if a father has an unhealthy lifestyle before a child is conceived, the epigenetics not only affects the father but may also affect the unborn child.

By Kristian Sjøgren

Obesity and unhealthy lifestyles can be passed from one generation to the next without any relation to childrearing. This concept is called epigenetics: molecular biological changes to genes that silence some genes and activate others.

Epigenetics plays a major role in conceiving and developing an embryo. For example, an egg cannot be fertilized with the cell nuclei from two females or two males, even though they are genetically compatible and could theoretically be the basis for developing an embryo. Instead, the cell nuclei from a male and a female need to be spliced to result in a fertilized egg. The reason is that epigenetics is gender specific, so even if the genes are compatible, the tiny molecular biological changes to the genes are not.

The latest research shows that epigenetics plays an alarmingly important role in developing an embryo – for both animals and humans. If a mother or father is obese, epigenetic changes can mean that their children will be predisposed to becoming obese even if they eat a normal diet. A child's risk of developing various lifestyle-related diseases also increases, and thus a father's unhealthy lifestyle at the time a child is conceived may affect a child for the rest of his or her life.

"Our research on the link between epigenetics and embryonic development focuses on understanding how various environmental factors, including food, exercise and obesity, influence embryonic development and a child's subsequent health," explains Romain Barrès, Professor, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Romain Barrès and his colleagues have published many scientific articles on this topic, including a review article in *Ugeskrift for Læger*, the journal of the Danish Medical Association.

Fat rats produce fat offspring

The researchers from the Foundation's Center for Basic Metabolic Research carried out one study involving rats fed a high-fat diet to make them obese. The hypothesis was that such a diet would alter the epigenetics of the reproductive cells, resulting in changes to their offspring.

The researchers fed male rats a high-fat diet for 8 weeks and then mated them with female rats that had been fed a normal diet. The males and females only had contact when they mated and were otherwise separated.

After the females gave birth, the researchers examined the offspring and compared them with the offspring of male rats that had not been fed a high-fat diet. What they discovered was that the young rats with overweight fathers metabolized glucose less efficiently than did the young rats with slim fathers. Inefficient glucose utilization is associated with developing obesity and is also a marker for developing type 2 diabetes.

The researchers speculated that the high-fat diet had triggered several epigenetic changes in the sperm cells of the male rats, which was also confirmed when they examined the rats' sperm.

"We found several epigenetic differences between the males fed a high-fat diet and those fed a normal diet. This proved that a father's diet can alter the health of his offspring and suggested that epigenetic changes in sperm cells may cause these changes," explains Romain Barrès.

Epigenetic changes to the genes regulating appetite and brain development

To support their findings, the researchers compared the epigenetic profiles of obese men with those of slim men. They discovered major differences in the epigenetic profile of the sperm cells.

In addition, the researchers discovered that the epigenetic changes occurred especially in the genes that regulate brain development and appetite.

"There were therefore good reasons to think that, if overweight influences the epigenetics of genes regulating appetite in sperm cells, then the children of overweight adults could have an altered regulation of appetite, for example, feeling more hungry," says Romain Barrès.

Epigenetics of sperm cells changes after weight loss surgery

The key question for the researchers then was whether making environmental changes can also alter epigenetics.

To examine this, the researchers collected the sperm from obese men who were scheduled to undergo gastric bypass surgery, which involves making a pouch at the top of the stomach so a person cannot eat as much before they feel full. The researchers collected sperm samples before and 1 year after the operation, when the trial participants had lost an average of 40 kg.

When the researchers compared the sperm samples, they again found epigenetic changes to the genes that regulate appetite and brain development.

"Several environmental factors clearly change the epigenetics of sperm cells. So far, we have not determined whether the epigenetic risk factors inherited by the next generation result from epigenetic changes in sperm cells from diet, overweight, exercise or other factors," explains Romain Barrès.

Exercise changes the epigenetics of genes regulating appetite and brain development

In a third study, the researchers examined how a 6-week training programme to improve fitness would influence the epigenetics of sperm cells of healthy young men.

The men were initially relatively unfit when they began and improved their aerobic capacity by 20%. In addition, the researchers again found epigenetic changes to the genes that regulate appetite and brain development.

"Why everything you do can change your epigenetics remains mysterious to us. Determining how manipulating epigenetics affects humans is not easy, but we are working to figure this out," says Romain Barrès.

Too early to offer dietary and exercise advice to future fathers

Realistically, does this mean that men should exercise, live healthily and lose weight before they try to conceive a child? According to Romain Barrès, the answer is both yes and no.

Romain Barrès first explains that there is no harm in telling men that at least they should be aware that not only a woman's health at conception influences the child's risk of developing disease or becoming obese. Men also need to take care of themselves before they start to have children.

"The idea that men only need to deliver sperm cells no longer applies. Men also pass on epigenetics that is influenced by how they live their lives," says Romain Barrès.

Nevertheless, the researchers cannot yet make recommendations in this field because the research still points in many different directions. For example, several animal experiments have shown that exercising before conception in males is associated with offspring predisposed with a risk of developing obesity.

"Exercise is a type of stress on the body, and the epigenetic changes may signal to the unborn embryo that it will have to store energy as much as possible to adapt to the increased needs, and this may upregulate the expression of genes that are responsible for appetite and for storing fat in the body. Too much exercise may therefore have counterproductive effects in the next generation," explains Romain Barrès.

Parents' overweight may be linked to a child's risk of autism

The link between epigenetic changes to the genes that regulate brain development and a father being overweight at conception is an extremely interesting discovery in its own right.

For example, the children of obese fathers have a 73% greater risk of being autistic.

Researchers from New York have studied the of sperm cells of the fathers of autistic children and discovered epigenetic changes to the genes that regulate both appetite and brain development.

"What is interesting is that the genes the New York researchers discovered when they examined the fathers of autistic children are 80% similar to the genes we have discovered. We therefore hypothesized that an unhealthy diet may result in epigenetic changes to sperm cells that may predispose a child to having an increased appetite and a higher risk of being autistic," says Romain Barrès.

Assisted reproduction may cause epigenetic changes

Finally, the researchers explain that these epigenetic changes that can result in various problems for children do not just apply to natural fertilization.

In assisted reproduction, the father and mother's reproductive cells are often exposed to many media containing both nutrients and vitamins, and this may also alter the epigenetics.

In addition, children conceived by assisted reproduction technology have an increased risk of several diseases, including cardiovascular diseases.

"Although assisted reproduction technology has enabled many couples to fulfil their dreams of becoming parents, emerging research in epigenetics suggests that this assisted reproduction technology should be constantly improved to minimize the risks for the next generation," concludes Romain Barrès.

"The importance of sperm epigenetics for conception and embryology" has been published in Ugeskrift for Læger, the journal of the Danish Medical Association. The last listed author of the article, Romain Barrès, is employed at the Novo Nordisk Foundation Center for Basic Metabolic Research.

What people ate 8,000 years ago

Analysis of protein residues in fragments of ancient ceramic bowls and jars reveals what was for dinner 8,000 years ago at the dawn of agriculture. The analysis confirms in unprecedented detail that early farmers in Anatolia ate a mixed diet of cereals, pulses, meats and milk products. In addition, they seemed to have knowledge of cultured dairy products 8,000 years ago.

By Kristian Sjøgren

When the early farmers sat down for dinner about 8,000 years ago, their food left protein residues in the vessels they used. A study of residues on the inside of fragments (sherds) of ceramic bowls and jars from the ancient settlement Çatalhöyük near Konya, Turkey has shown for the first time that food protein can remain preserved in bowls and jars for such a long time.

The analysis also shows that these early farmers processed or served milk products from sheep, goats and cows in these bowls besides wheat, barley, peas and vetch as well as meat, mostly from sheep and goats, but also from cows and deer. Further, they may have prepared meals, presumably in the form of porridge or soup, in the ceramic vessels.

The discovery provides the most detailed picture so far of people's diet during this era of human history. So far, researchers have been able to learn about prehistoric diets from refuse such as animal bones and plant remains and by analysing the fat preserved in ceramic vessels. Now, using this new technique, researchers can obtain a much greater level of detail about food, analysing the specific grains, plants and animal species found in the prehistoric bowls and jars.

"Although bones from various animals have previously been found in the settlements as well as traces of milkfat in vessels, this is the first time we have evidence that all three ruminant species known from the bone finds – sheep, goats and cattle – were used for both meat and milk," explains a lead researcher behind the study, Eva Rosenstock, Institute of Prehistoric Archaeology, Freie Universität Berlin, Germany.

The study has been published in *Nature Communications*.

One of the world's best-preserved prehistoric settlements

Çatalhöyük is a large Stone Age settlement inhabited between 9,100 and 7,600 years ago. The settlement is located in central Turkey and is incredibly well preserved, with houses built next to one other.

After 25 years of excavation, Çatalhöyük is also one of the most thoroughly analysed agricultural settlements from the era of the earliest farmers.

For the study, archaeologists collected and analysed residue deposits on fragments of ceramic bowls and jars from a narrow time frame ranging from 7,900 to 7,800 years ago: the last flourishing period on the site of Çatalhöyük.

These fragments were collected from the West Mound of the settlement and contained deposits similar to limescale on the inside of what were once jars or bowls.

"The eastern part of the settlement yields about one vessel fragment per bucket of excavated soil. About 8,000 years ago, people moved to the West Mound of the settlement, and there the number of vessel fragments explodes – dozens of pieces per bucket of soil," says Eva Rosenstock.

Proteins from a wide variety of foods

The researchers analysed the deposits on the inside of the vessel fragments. Archaeologists often ignored or even remove these deposits as postdepositional sinter, but the new study shows that the dirty dishes of the Stone Age farmers contain an absolute gold mine of information.

The researchers analysed the deposits for the presence of both protein and fat based on the theory that this residue must originate from the foods the farmers stored in them.

These proteins are especially interesting because most are species-specific. The researchers used shotgun proteomics, an advanced analysis technique that is used to identify the protein sequences of all the individual proteins at the same time, to determine the organisms and tissues from which the proteins originated. They looked up the proteins found in the jars and bowls in a database containing thousands of protein references from animal and plant species.

They found proteins from wheat, barley, peas, goat, mutton, milk and other foods in the bowls and jars and found several foods stored in the same vessel, so the vessels might have been used to prepare, consume or even store meals, but different foods could also have been stored in a specific vessel but not at the same time.

Expanding the database to improve insight

The database of proteins is not complete, which means that the researchers think that these first farmers ate other foods than those identified so far in their analysis.

Various circumstances may explain why researchers have not found traces of these other foods.

- The food was stored elsewhere than the ceramic bowls and jars.
- The protein databases do not contain the proteins.

"For example, we have only six protein sequences for vetch in our database versus 145,000 protein sequences for wheat. This makes identifying wheat easy but identifying vetch difficult," explains the main author of the study, Jessica Hendy, Department of Archaeology, Max Planck Institute for the Science of Human History, Jena, Germany.

Jessica Hendy also explains that expanding the protein database is an important part of the future work of examining our ancestral diets.

A message for colleagues: keep the deposits on vessel fragments

The archaeologists made an especially interesting discovery: one of the vessels, the jar, only contained proteins from whey, which indicates that these early farmers separated milk from sheep, goats and cows into whey and curds.

"This shows that people in that era used dairy techniques to separate the milk into its various components and that they subsequently stored the whey for some unknown purpose," says Jessica Hendy.

According to Eva Rosenstock, the study shows how protein analysis can be a very powerful tool in improving insight into how people lived in the past.

However, calcitic residue on ceramic fragments should not habitually be treated with acid, because this may remove the material needed for analysis. Eva Rosenstock has a message for colleagues.

"These results highlight how valuable these deposits are, and we encourage our colleagues to keep them on the fragments during processing and cleaning after archaeological excavations," says Eva Rosenstock.

"Ancient proteins from ceramic vessels at Çatalhöyük West reveal the hidden cuisine of early farmers" has been published in Nature Communications. Researchers from the Novo Nordisk Foundation Center for Protein Research, University of Copenhagen are co-authors.

Vigorous exercise can suppress appetite

A Danish study indicates that vigorous-intensity exercise suppresses appetite, but the effect only lasts for a few months. The study is the first of its type to examine how long-term exercise affects appetite among individuals with overweight or obesity.

By Kristian Sjøgren

You might feel that your appetite declines when you increase the intensity of your workouts.

Actually, this may be true, and a new Danish study shows that 3 months of vigorous exercise training suppresses appetite.

This sounds like good news, but unfortunately, the appetite-suppressing effect of exercise does not seem to last and the study shows that the appetite suppression wanes after 6 months.

"The interaction between exercise, appetite and weight loss is very complex. We do not know what the long-term effects of exercise training on appetite regulation are. However, this study begins to lift the veil on the underlying mechanisms," says Jonas Salling Quist, who carried out the study with other researchers as part of his PhD study at the Department of Biomedical Sciences, University of Copenhagen.

The study has been published in the *Journal of Applied Physiology*.

Last author Mads Rosenkilde was a postdoctoral fellow during the study, which was part of GO-ACTIWE, a randomized controlled trial led by Bente Stallknecht, Professor, Department of Biomedical Sciences, University of Copenhagen.

How exercise affects overweight people
The study is one of a series of studies in which the researchers from the University of Copenhagen have studied how exercise training affects various parameters, including appetite, weight loss and various biological markers in the blood, including the concentration of gastrointestinal hormones.

This randomized controlled trial randomly distributed 130 physically inactive women and men who were overweight or obese into four groups:

- a control group that continued their usual lifestyle;
- an exercise group that volunteered to commute by bicycle;
- a group that volunteered to perform leisure-time exercise at moderate intensity; and
- a group that volunteered to perform leisure-time exercise at vigorous intensity.

The participants in the three exercise groups were asked to wear a heart rate monitor during all sessions and were instructed to exercise five days a week and burn 320 calories per day for women and 420 for men.

Participants in the cycling group who could not burn the specified number of calories because they lived too close to their workplace were asked to make a detour to increase the distance. Conversely, participants who lived further away were asked to combine cycling and public transport.

"This was necessary to ensure that the energy expended during exercise was similar in the three exercise groups. This situation is somewhat artificial, and one must be aware of this when evaluating the results and the extent to which they can be generalized," says Jonas Salling Quist.

Examining how much people ate
The researchers examined the participants' appetite before the study started and 3 and 6 months later. Appetite was assessed based on self-rated appetite and the concentrations of biological markers in the blood associated with:

- eating a standardized breakfast;
- exercising on a stationary cycle; and
- how much the test participants ate during an ad libitum meal served at the end of the test day.

"Previous studies have primarily been shorter and without a control group. This is the first study to investigate the effects of different types of exercise over longer periods of time with repeated measurements and to compare the effects with a control group," explains Jonas Salling Quist.

Vigorous exercise temporarily suppresses appetite
After 3 months, participants in the vigorous-intensity group reported less appetite after the standardized meal and after exercising on the stationary cycle compared with the control group. These participants also consumed food with 22% less energy at the subsequent ad libitum meal than the control participants did.

The appetite-suppressing effect was restricted to the vigorous-intensity exercise group.

The exercise intensity was not specified for the commuting participants, but they cycled on average at moderate intensity. The results therefore suggest that exercise must be vigorous to suppress appetite.

"Previous studies have shown that a single exercise session at vigorous intensity, such as on a stationary cycle, acutely reduces appetite and alters the concentration of gastrointestinal hormones in a way that indicates a biological appetite-suppressing effect. However, we did not find significant changes in biological markers that could explain the suppressed self-rated appetite and energy intake among our participants. When participants in our studies change from being physically inactive to suddenly exercising intensively, it affects their energy balance and leads to fat loss. However, the body does not seem to compensate for this by increasing appetite, as has been observed in diet-induced weight loss, for example," says Jonas Salling Quist.

Weight loss declined after 3 months
In the same study, the researchers examined how the three different ways of exercising affected participants' energy balance and fat loss.

All three groups lost body fat but mostly during the first 3 months, and then the fat loss declined from 3 to 6 months.

"We cannot conclude that there is an association between changes in appetite and fat loss in our study, but we cannot exclude an association either. However, our study suggests that something happens in the body when people with overweight and obesity exercise at vigorous intensity for 3 months and that this affects both appetite and weight. The participants continued to exercise from 3 to 6 months, but they compensated for the energy used in one way or another, and this could include small changes in energy expenditure outside the exercise sessions and in free-living energy intake," says Jonas Salling Quist.

Further research to improve exercise programmes for people with obesity
After 6 months, the effects on self-rated appetite and ad libitum energy intake had disappeared, but concentrations of the GLP-1 hormone were higher in the fasting state and after the standardized breakfast among those exercising vigorously compared with the control group.

GLP-1 has an appetite-suppressing effect and therefore has an important role in regulating appetite, but the GLP-1 concentration apparently was not clearly associated with reduced self-rated appetite and energy intake.

Jonas Salling Quist says that the conclusions based on the study should be treated with caution because it was only exploratory. There is considerable variation in how exercise affects appetite and energy balance, and future studies should be dimensioned to take these factors into account.

"We can conclude that vigorous exercise appears to temporarily suppress appetite among people who were previously physically inactive and overweight or obese. The strengths of our study include the long trial period with repeated measurements and comparing the effects with a control group. Future studies should probe more deeply into understanding the complex interaction between exercise and appetite and how this knowledge can be used to create better exercise training programmes for overweight people, who are at risk of developing lifestyle-related diseases such as diabetes and cardiovascular diseases," says Jonas Salling Quist.

"Effects of active commuting and leisure-time exercise on appetite in individuals with overweight and obesity" has been published in the Journal of Applied Physiology. Jens Juul Holst, a co-author, is a Professor and Scientific Director at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Natural hormone can make you choose salad instead of candy

Researchers made a startling discovery a few years ago: the liver, which secretes a hormone called FGF21, controls people's sweet tooth. FGF21 strongly suppresses our desire to consume sugar and alcohol. However, translating this knowledge into medicine that can treat overweight people with sugar cravings requires that researchers understand why evolution has provided us with a hormone that inhibits the appetite for sugary sources of energy. Evidence suggests that FGF21 resulted from our ancestors' consumption of fermented fruit and alcohol.

By Morten Busch

The epic internal battle people experience between eating a healthy salad versus a sweet cake is well known, and the cake is increasingly winning for now. Researchers have therefore focused on determining why we choose unhealthy food rather than healthy food. Glands, fat cells or the digestive system secrete most of the known hormones that regulate hunger and appetite. The discovery that the liver secretes fibroblast growth factor 21 (FGF21), a hormone that very specifically regulates our desire to consume sugar and alcohol, was therefore both surprising and promising.

"Increasing the concentration of FGF21 in mice halves their consumption of sugar, and removing it entirely makes the mice almost uninhibited in ingesting sugar and alcohol. We therefore believe that FGF21 has evolved to protect our ancestors from consuming excessive amounts of fermented fruit and alcohol. Now we hope that FGF21 can help people in combating overweight and obesity if we can develop it into something that can inhibit the craving for sugar," explains Matthew P. Gillum, Associate Professor, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Consume twice as much sugar if the gene is missing

Since research discovered the remarkable function of FGF21 in 2016, many studies have been carried out to understand how it works and especially whether it has the same effects on people as on rodents. The researchers have now collated all these results and knowledge into a review article in the Journal of Physiology.

"In 2016, research showed that FGF21 selectively inhibits mice's consumption of sugar and especially alcohol, which develops when sugar-laden food such as fruit ferments. But FGF21 does not affect their intake of fat, protein or more complex carbohydrates. Since then, researchers have demonstrated the same effect among people, especially for consuming alcohol."

Genetic studies have even revealed that the quantity of candy and alcohol people consume varies with changes in the gene encoding FGF21, which suggests that the natural variation in the amount of FGF21 each person secretes explains differences in people's appetite for sugar and the fermented products of sugar.

"Increasing the concentration of FGF21 in mice halves their consumption of sugar, and removing it entirely makes the mice almost uninhibited in ingesting sugar and alcohol."

Matthew P. Gillum

"We still do not know exactly which changes have which effects, but we can artificially reduce mice's appetite for sugar – either by injecting FGF21 into the bloodstream or by genetically overexpressing the gene encoding for it. If we remove the gene completely from these mice, then they consume about twice as much sugar and alcohol as normal."

Defence against fermented fruit

The latest research emphasizes that improving the understanding of how hormones interact in the body is crucial for solving the growing challenge in high-income countries related to the numerous complications the obesity epidemic causes such as type 2 diabetes, non-alcoholic steatohepatitis, cardiovascular disease and cancer.

"The big question we need to answer to meet this challenge is how these hormones interact with the brain. For example, how do the strong signals we get from our taste buds when we eat sugar interact with how the brain otherwise realizes which types of food provide energy and the building blocks the body needs? It is quite obvious that evolution deceives us in influencing the food we eat now compared with what our ancestors ate back then."

One of the researcher's theories about the function of FGF21 is based on how the world was once. The liver seems to have a negative feedback mechanism so that consuming sugar or alcohol increases the secretion of FGF21 shortly afterwards.

"We believe that FGF21 is the body's attempt to protect against the toxic effects on the liver resulting from, for example, consuming excessive fermented fruit, which was an important food source for our ancestors. Presumably, therefore, people's livers respond to excessive intake of sugar and alcohol by producing a hormone that reduces appetite for alcohol and sugar."

It is still too early to confirm this theory, but the researchers are busy investigating how differences in the concentration of FGF21 influences people's appetite. In addition, the researchers plan to repeat the experiments that have previously been done on mice but using the alcohol-consuming vervet monkeys from Saint Kitts in the Caribbean.

"Of course, the ultimate question is why we still consume so much sugar despite producing FGF21, which should inhibit our appetite for sugar. Does this result from genetic changes that reduce the secretion of FGF21or from other factors in the interaction between hormones? If we succeed in finding these connections, we can probably also find a way to influence the balance so that we learn to resist the temptations of sugar – and thereby may increase our appetite for healthier food and avoid becoming overweight and developing fatty liver."

"FGF21: an endocrine inhibitor of sugar and alcohol appetite" has been published in the Journal of Physiology. Matthew P. Gillum is an Associate Professor at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

In the body's engine room

Content

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Flippases give cells an electric shock
New research shows that phospholipid flippases create an electric current as these proteins move molecules of fat in the cell membrane. These flippases strongly influence the development of certain diseases.

Uunderstanding how fetuses develop may cure diabetes
Researchers have mapped how the pancreas is formed at the fetal stage. This is a step on the way to being able to create a pancreas from scratch to help to cure diabetes.

Micromotors can ferry living cells
Researchers have invented real-time hydrogel-producing micromotors that can entrap and move cells. This discovery presents significant pharmaceutical opportunities and the potential to improve how tissue can be designed both outside and inside the human body.

New study sorts out how the destiny of cells is determined
Both inheritance and environment determine a person's fate, but what determines the fate of a cell? Does inheritance predetermine the fate of a cell at birth or does its fate depend on the environment in which a cell develops? This knowledge is vitally important for inducing stem cells to differentiate into certain types of cells and has been the subject of many scientific discussions. Now researchers have found an answer, which might ultimately help treat both cancer and diabetes.

Researchers find a key to understanding how cells remember what type they are

When cells divide, they do not merely replicate the genome. The molecules bound to the genome define the cell's function and must also be replicated; otherwise, diseases such as cancer can develop. This extremely complex mechanism has been almost impossible to characterize, but researchers have now discovered a key to understanding the process. Just one error in a protein entirely ruins the copying process. This discovery could be decisive in understanding cell fate decisions in development.

By Morten Busch

The human body comprises more than 200 known types of cells, each with its own function. The cells contain exactly the same genetic code but differ based on the epigenetic signature. For example, methyl groups can bind to the DNA and thereby silence genes. Similarly, proteins called histones package our metre-long chromosomes and ensure that genes, including the cell type–specific ones, are correctly regulated. When cells divide, this information must be passed on to the new daughter cells.

"The epigenetic signature can be viewed as a cell's memory. Until now, we have known in detail how the genome is replicated, but it has remained unknown how the epigenome is replicated. Our latest study reveals how histones, building blocks of the cellular memory, are transferred when a cell divides. We have shown that the MCM2 protein is key for transferring these building blocks and thus propagating epigenetic cell memory. This breakthrough will be instrumental to fully understanding cell memory, a fundamental biological process important for developing a healthy organism that counteracts diseases such as cancer," explains Anja Groth, Professor, Biotech Research & Innovation Center, University of Copenhagen.

Tiny change with big effects

A pioneering new technique has enabled the researchers to identify the protein that ensures that histones are inherited when cells divide. The new technique, sister chromatids after replication (SCAR) sequencing, enables researchers to track proteins bound to the two new DNA strands created when DNA is replicated. Using this technology, the researchers identified a key role for the protein MCM2 in distributing histones and the information they carry to both DNA daughter strands.

"This provides the first direct evidence that a specific protein is directly linked to transmitting histone-based information and thus replicating the cell's epigenetic profile, which constitutes the memory about what type of cell it is."

The experiments showed that MCM2 is responsible for correctly distributing histones during DNA replication, ensuring that they are transferred to the new cells. Histones and the genome create a structure called chromatin, and this structure helps cells to maintain the correct functions.

"MCM2 is therefore directly linked to the replication of histone-based information. When we mutated MCM2 so that it could no longer bind histones, the histones were no longer distributed to both DNA strands. This provides us with a unique system to investigate what happens with cell memory and organismal development when the inheritance of information in histones is disrupted."

Unlocking many new opportunities

Because many cells in the human body divide throughout life, it is critical to understand how they remember what they are and whether they should give rise to skin, liver or intestinal cells. This is important for developing and maintaining a healthy organism and to avoid diseases such as cancer. However, the mechanisms that govern epigenetic cellular memory are unclear, and this continues to perplex researchers.

"A recurring question has been the extent to which the chemically modified histones are randomly transferred during DNA replication. Our study shows that this process is tightly controlled and not random. With our new knowledge and uniquely engineered cells, we can begin to examine exactly how the inheritance of this information affects cells and the development of whole organisms."

This new ability to block the proper handling of histones enables the researchers to determine exactly how important histone inheritance is for developing a complete organism from a single cell: that is, whether disturbing the function of MCM2 influences the ability to create other types of cells.

"Researchers often discuss how important the information histone contains really is for a cell's identity and fate. This discovery enables us to start new experiments that may finally answer this question. So this is an incredibly important key to unlocking many longstanding enigmas."

"This is an incredibly important key to unlocking many longstanding enigmas."

Anja Groth

This new understanding of a key mechanism in histone inheritance provides new opportunities within stem cell research and regenerative medicine, such as taking skin cells from people and reprogramming them. It can also help understanding how perturbing cell fate contributes to cancer.

"When cancer develops, cells change their identity and obtain unwarranted properties. The fact that cells remember what type they are and thereby their function helps to prevent them from developing undesirable characteristics such as dividing unimpeded, which cancerous cells do. We know that cancer cells suffer pervasive changes in chromatin, and it will be exciting to investigate how impaired transmission of histones influences cancer predisposition."

"MCM2 promotes symmetric inheritance of modified histones during DNA replication" has been published in Science. The research was funded by the Independent Research Fund Denmark, the European Research Council, the Lundbeck Foundation and the Novo Nordisk Foundation.

Help! Why do our cells have so much RNA with no apparent function?

A cell nucleus produces significantly more non-functional RNA than functional RNA. A Danish research group is delving deep into the engine room of evolution to try and find the reason why.

By Kristian Sjøgren

Your cells are full of RNA with no apparent function. In fact, researchers can only account for the function of 2–3 % of the RNA produced by the genome. The function of the remaining RNA, if any, is unknown.

Even though the apparently useless RNA has no immediate function, it can still affect our health. An ingenious system of different protein complexes protects and degrades both the functional RNA and the apparently useless RNA. If mutations arise in some of these protein complexes, this can lead to the development of cancer, disorders of the nervous system or other diseases.

This is why researchers are extremely interested in discovering how this non-coding RNA that does not translate into proteins affects the cells and, ultimately, the well-being of the organism and how the body's cells regulate what RNA to keep and what to destroy.

"Researching these cellular processes means researching the fundamental processes of how our genome works. It is the lifeblood of evolution and biology, and it is easy to imagine that if these processes do not function properly, the cells will be flooded with non-coding RNA that can cause all kinds of problems," says Torben Heick Jensen, Professor, Department of Molecular Biology and Genetics, Aarhus University, who leads an extensive new research project in this field.

The Novo Nordisk Foundation recently awarded Torben Heick Jensen a major Challenge Programme grant. For the next 6 years, he and colleagues from the University of Southern Denmark in Odense and the Max Planck Institute of Biochemistry in Martinsried, Germany will be busy performing experiments and delving deep into the engine room of biology.

Only a few percent of the function of the body's RNA is accounted for

Torben Heick Jensen and his research group will basically be trying to determine how cells decide whether RNA should be kept or destroyed.

RNA enables DNA to function. Each of the genome's genes is an architectural blueprint of a protein, and the body's cells create pieces of RNA from the gene – messenger RNA (mRNA) – that can be translated into proteins.

Other pieces of RNA, such as transfer RNA (tRNA) and ribosomal RNA (rRNA), have structural roles and support the translation process even though they are not translated into protein.

And then there is the remaining RNA, the function of which still baffles researchers.

"Many types of non-coding RNA have various functions. However, this non-coding RNA is still important. If we add up all the RNA of which we know the functions, we can only account for a mere 2–3% of our genome," explains Torben Heick Jensen.

Errors in regulating RNA can lead to cancer

The body's cells are constantly performing a delicate balancing act to determine which RNA can be used and which cannot, and if the body's cells do not maintain good RNA hygiene, then things can go wrong very quickly.

For this purpose, the cells are equipped with various protein complexes that can preserve or degrade pieces of RNA in the cell nucleus. If these protein complexes are defective, such as from a mutation, the cell almost immediately loses its ability to function properly.

The reason is that RNA – both the functional and the non-coding RNA – are continually produced, and if they are not sorted, the apparently useless RNA binds to proteins that it should not bind to, and the most basic cellular functions cease.

If this happens, a cascade effect begins that will ultimately affect people's health. This is why mutations in the proteins that ensure good RNA hygiene are also linked to various diseases, such as cancer and disorders of the nervous system.

"RNA biology plays a big role in neurons. This is where we often see diseases in connection with mutations in the RNA degradation complexes, but we cannot say for certain that there is 1:1 relationship between mutations and specific diseases," says Torben Heick Jensen.

Nevertheless, research into the cellular RNA degradation complexes opens up a variety of pharmaceutical opportunities.

Torben Heick Jensen envisages that using the gene editing technology CRISPR could, for example, correct errors in the complexes that degrade RNA, thereby preventing people from developing disorders of the nervous system and cancer.

"We are not researching this ourselves, but these are the prospects, and other people are investigating the potential of this field," he says.

A pioneer in discovering new RNA

Torben Heick Jensen and his team are researching the protein complexes that degrade the RNA with no apparent function.

As of 10–15 years ago, researchers believed that most of the genome was not transcribed into anything useful, and certainly not to non-coding RNA, but Torben Heick Jensen's research and that of other researchers show that this happens.

In 2008, the research team at Aarhus University published an article in Science outlining an experiment in which they shut down part of the RNA degradation machinery. The cell produced enormous amounts of RNA, which was previously unknown, since it had been degraded as rapidly as it was being produced.

"There was an explosion of new RNA that had never been seen before. We were co-pioneers in discovering that cells contain much more RNA that we had previously imagined. The main challenge in this field now is to determine exactly why the cells transcribe the genome into RNA that has no apparent function," says Torben Heick Jensen.

RNA can make evolutionary sense

So why does evolution enable a cell to use its valuable resources to create RNA that has no function and is immediately degraded? This seems like such a waste.

But it actually makes sense to Torben Heick Jensen from an evolutionary viewpoint.

According to Torben Heick Jensen, the constant production of RNA brings life to the genome. This makes the genome become a dynamic toolbox that contains many potential new tools that could have a function in the future or tools that had a function in the past.

Some pieces of RNA could one day be used to encode a protein that benefits the cell or the organism in some way. Unless the RNA is expressed, the cell can never test these potential possibilities. In this case, it would only have access

to the genome, which is like having a drawing of a hammer rather than an actual hammer. Determining whether or not a hammer is useful is difficult unless you test it in the real world.

"Having a dynamic genome with which to test things makes perfect sense in evolutionary terms. It does, however, require you to have the necessary machinery to maintain a certain level of hygiene," says Torben Heick Jensen.

Protein complex degrades RNA
The specific degradation complex that Torben Heick Jensen previously deactivated in his research is called the RNA exosome.

The RNA exosome destroys RNA by indiscriminately degrading the RNA building blocks. Most RNA exosomes are present inside the cell nucleus, where the genome is translated into RNA, so if the RNA is to become the template for creating a protein, it must exit the cell nucleus as quickly as possible before an RNA exosome gets hold of it and degrades it.

The cell nucleus is a harsh environment for the RNA, but the cytoplasm outside the nucleus is a safe haven.

To assist the functional pieces of RNA in escaping the hellish environment of the cell nucleus and to prevent the non-coding RNA from exiting, evolution has equipped the cells with a series of protein complexes with various functions.

In addition to RNA exosomes, other protein complexes bind to the exosomes and help them to identify the correct RNA to break apart: adaptor complexes.

Cells also have protein complexes that bind to specific RNAs and protect them from exosomes while guiding the RNA out of the cell nucleus. These are the pieces of RNA that will be translated into functional proteins later.

The interaction between the protein complexes therefore determines which pieces of RNA escape from the cell nucleus and which ones are degraded as quickly as they are produced.

"The complexity of the whole system is enhanced by the fact that some RNA has a function in some cells and not in others. Each cell must therefore be equipped with specific protein complexes that ensure that the right pieces of RNA are expressed as proteins in the cytoplasm of a specific type of cell whereas others are not."

Torben Heick Jensen

Discovering two protein complexes that decide the RNA's fate
In relation to the present project, Torben Heick Jensen has had two major highlights in his research career. In 2011 and 2016, respectively, his research group identified two adaptor protein complexes: the proteins that help exosomes recognize the pieces of RNA to be degraded.

These adaptor complexes sort through the RNA for the exosomes and hand over the pieces of RNA to be degraded.

Torben Heick Jensen has just been awarded a grant to perform further research into identifying and mapping adaptor complexes.

In addition to mapping the precise structure of the complexes that have already been identified, Torben Heick Jensen hopes that his research team will also discover new adaptor complexes.

"There may be 5–10 adaptors in all. This depends somewhat on how broadly one looks. There may be some in human cell lines, and others may be in stem cells. This is because stem cells have a slightly different biology and need a different RNA expression to remain pluripotent and to continue to have the ability to differentiate into different types of cells. Finding one or two new adaptor complexes during the 6-year project would be a major achievement," says Torben Heick Jensen.

Mapping adaptor complexes in minute detail
If the researchers discover new adaptor complexes, they will work on mapping their structures. They will start off by doing this with the two adaptor complexes they have already discovered.

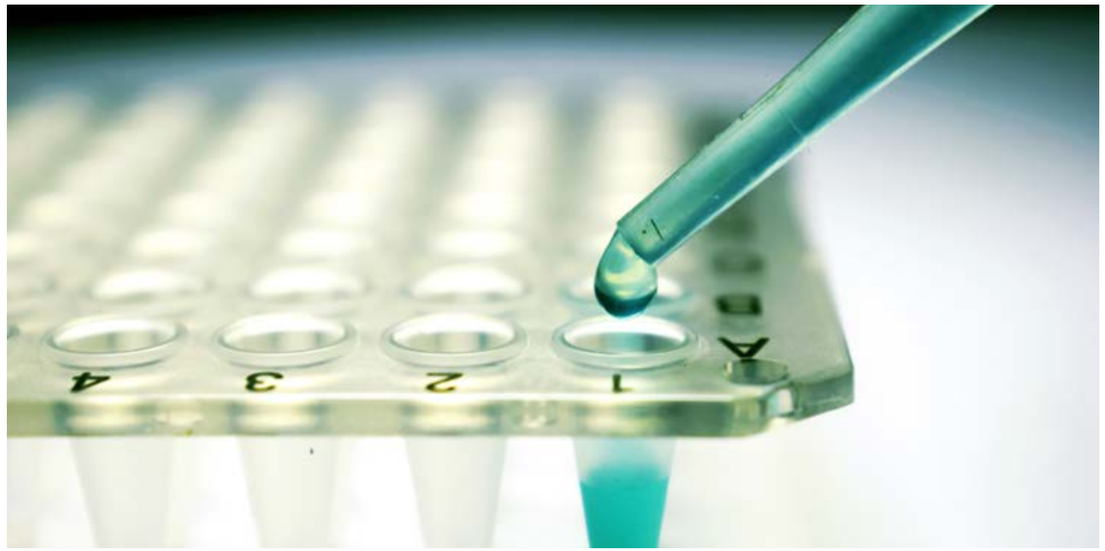
When the researchers map these structures, they first find the genes that create the proteins in the protein complexes. Then they express the genes in a bacteria, yeast or mammalian cell, so they can produce enough proteins to do further studies.

When Torben Heick Jensen and his colleagues have successfully produced large amounts of adaptor proteins, they must try to get them in crystal form so they can X-ray them.

This technique is known as X-ray crystallography. How the proteins scatter the X-rays reflects the structure of the specific protein.

Parallel to this, the researchers will also study the proteins by taking hundreds of images with an electron microscope.

This technique is called cryoelectron microscopy, in which researchers take the many indistinct images of a protein and then consolidate the images into one sharp image of the protein using advanced imaging software.



Using the protein to discover more adaptor complexes
The research project is already underway, and the researchers from Aarhus University, the University of Southern Denmark and the Max Planck Institute of Biochemistry have mapped some very interesting structural aspects of the two adaptor complex structures that they can potentially use to find other ones.

Both protein complexes have a part that is important to the functioning of the exosome. This is called MTR4, and Torben Heick Jensen envisages that, if two adaptor complexes have this and therefore can optimally interact with the exosomes, then other potential adaptor protein complexes may also have them.

"If this is the case, then we can use MTR4 to find other adaptor complexes. This is the approach we are taking," says Torben Heick Jensen.

RNA structure determines whether it is destroyed
Mapping the adaptor complexes that have already been discovered and possible discoveries of new ones will give researchers a much clearer idea of what happens inside cell nuclei when RNA is being created, degraded or protected.

Torben Heick Jensen envisages a very dynamic fate for all the pieces of RNA.

When RNA is being produced, the adaptor complexes recognize it within milliseconds, but the interaction with an adaptor complex is not static.

Adaptor complexes continually bind and release the RNA, maintaining an equilibrium between the exosomes that want to destroy the RNA and the proteins that want to help the RNA safely exit the cell nucleus.

"The RNAs that exit the cell nucleus generally contain fewer nucleotide sequences, known as introns, than the pieces of RNA that are not exported from the cell nucleus. This points to a mechanism that cells use to differentiate between the functional pieces of RNA and the non-coding pieces. We do not completely comprehend the entire recognition process, but we will get there," explains Torben Heick Jensen.

RNA may have an unknown effect
Overall, the research should improve understanding of whether the apparently useless RNA has other functions than being a dynamic toolbox for evolution.

Torben Heick Jensen's research has shown that RNA has a very short lifespan inside the cell nucleus, but part of the RNA may still have a function in the short time it exists before it is degraded.

"Right now, we think that much of it is just produced so it can be degraded again, but some of it may well have a transient effect that we would also like to characterize," says Torben Heick Jensen.

"The MTR4 helicase recruits nuclear adaptors of the human RNA exosome using distinct arch-interacting motifs" has been published in Nature Communications. "Escaping nuclear decay: the significance of mRNA export for gene expression" has been published in Current Genetics. "Controlling nuclear RNA levels" has been published in Nature Reviews Genetics. In 2018, the Novo Nordisk Foundation awarded Torben Heick Jensen a Challenge Programme grant for the project Function, Structure, Regulation and Targeting of Exosome Adaptor Complexes.

How cells decide their fate as a fetus develops

New research shows how a specific type of stem cell decides to become facial bone cells, nerve cells, pigmented or mesenchymal stem cells as a fetus develops. This discovery could be important for regenerative medicine and for understanding how some types of cancer develop.

By Kristian Sjøgren

Neural crest cells are a very plastic type of cell that exists during early embryonic development in the same domain that will give rise to the spinal cord. They generate numerous types of cells, including nerve cells, pigmented cells, facial bone cells and muscle cells. Now, for the first time, researchers have found out how these cells decide on the role they will take. The research shows that the cells must continually decide whether to move in the direction of becoming one specific type of cell. If a cell does not do this, it has the opportunity to develop into another type of cell.

This discovery is important in understanding how different types of tissue develop as the fetus develops and may help in better understanding some types of cancer in which the decision-making process towards becoming one type of cell or another malfunctions.

Ultimately, the discovery may also help in developing new approaches in regenerative medicine, in which doctors in the future could make replacement tissue in the laboratory and transplant it into people who have lost their hearing, for example, or have had sections of their intestines removed as a result of severe infection.

"Basically, we asked ourselves: 'How do cells know what they will become?'. Our study answered this question. We can now see where the cells come from and what decisions they make to become several types of specialized cells," explains a co-author, Maria Eleni Kastriti, Postdoctoral Fellow, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.

The new study was recently published in *Science*.

The environment determines the fate of cells
Neural crest cells can develop into many types of cells: bone cells in the ocular cavities, bone cells in the ears, jaw and teeth, glial cells that protect nerve cells, skin pigment cells, nerve cells in the head just outside the skull and nerve cells in the autonomic and enteric nervous systems.

When neural crest cells develop towards a particular type of cell as the fetus develops, the cells migrate to the location in the body where they are needed.

Maria Eleni Kastriti's research shows that the cells are not preprogrammed to become any specific type of cell. Instead, the cells are induced to develop in one direction or another as they migrate.

The environment during migration determines how the cells develop: for example, a neuron-derived signal in the base of the brain influences the first migrating cranial neural crest cells to become precisely bone cells.

Researchers hypothesize that growth factors in the environment of the neural crest could influence groups of genes that determine the fate of each individual cell. For example, one group of genes might promote a cell to become a nerve cell and another group of genes tries to maintain the cell in the undifferentiated state.

"Different groups of cells constantly interact, and the gene expression within neural crest cells oscillates without the cell finally deciding its fate. However, when the environment is right, the gene expression is steered in one direction or another and then the cell begins to transform," explains Maria Eleni Kastriti.

Cell decisions are like driving on a motorway
A key discovery in the new research is how the cells decide whether to become one type of cell or another.

There are three different hypothetical scenarios.

- The cells are preprogrammed to develop into certain types of cells.
- The cells have to decide to go in one direction of many, and each of these directions opens up new opportunities to further specialize in the direction of one type of cell or another.
- The cells have to decide whether to develop into one type of cell, and if a cell does not do this, it has the opportunity to develop into another type of cell.

The new research shows that the cells follow the third option.

"Like driving on a motorway, there is always a new exit that leads somewhere. This is also how the cells develop. Once they have passed an exit, they cannot develop into that type of cell, and then they have to make the choice to become the next type or the one after," says Maria Eleni Kastriti.

An interesting aspect of this conclusion is that the cells' first possible decision is whether to develop into neurons in the sensory organs and the last possible option is whether to develop into neurons in the autonomic nervous system that regulates breathing and heart rate, among other involuntary functions.

"Interestingly, a cell's first and last options are to develop into a neuron, with the decisions on the other types of cells in between," says Maria Eleni Kastriti.

Studying how cells develop in mouse fetuses
Maria Eleni Kastriti and her colleagues studied gene expression in mouse fetuses.

They used various genetic engineering techniques to measure the activity of various groups of genes on the single-cell level and determined how cells interact genetically in different locations in the bodies of the mice and at different times as the fetus underwent early development.

Based on these observations, they proposed a hypothesis on the options facing neural crest cells as the fetus develops.

Making specialized intestinal cells in the laboratory
The discovery has several interesting perspectives that may become clinically relevant in the future.

For example, some people have such a severe stomach infection that doctors have to remove some of their intestines.

In this situation, the doctors would like to be able to replace the intestinal tissue they removed with new tissue. However, cultivating artificial tissue in the laboratory requires that doctors know how to influence the cell culture environment so the cells can develop into exactly the cells needed to construct a new section of the intestines. Creating a functional enteric nervous system in vitro is especially demanding. This is absolutely necessary for any intestinal transplant to be functional. This research provides important insight into how cells derived from the same patient could be used and reprogrammed to colonize the manufactured tissue, leading to fully functional intestinal transplants.

One can also imagine a similar situation for people with impaired hearing when the specialized neurons inside the ear have been damaged. The damaged tissue can also be replaced, but only if the cells can be induced to develop in the direction of the missing type of cell.

"This also applies to people with certain congenital diseases in which the development of certain types of cells originating from the neural crest cells malfunctions. Our discovery can help to identify where things go wrong and can help shape the direction for developing therapy that might repair the damaged tissue. Neural crest cells are present in the skin, and the cells that are missing can be created from a skin biopsy, but only if you know how to influence them to develop in the right direction," explains Maria Eleni Kastriti.

Improving knowledge about cancer
Another field in which this discovery may be interesting is improving our understanding of how some types of cancer develop.

The development of some types of cancer, such as paraganglioma or pheochromocytoma, cancer in the body cavity close to the kidneys, involves more than one type of cell.

The evidence suggests that the cancerous event could originate from a time before the cells differentiated, dating back to the stage of the neural crest cells

With the overview of the different stages of cellular development, the researchers can now determine at what point during fetal development any malfunction may have arisen.

"Much research is already being carried out in this field because it will significantly improve understanding of how several types of cancer develop that we do not know much about today and how different types of cancer in the periphery of the body can be so fundamentally heterogeneous," explains Maria Eleni Kastriti.

"Spatiotemporal structure of cell fate decisions in murine neural crest" has been published in Science. In 2017, the Novo Nordisk Foundation awarded a grant to co-author Maria Eleni Kastriti for the project Profiling of Neuroendocrine Cells and Novel Cell Types in the Human and Mouse Adrenal Gland.

Surprising breakthrough: All immature cells in the intestine can become stem cells

Stem cells have an unresolved potential for treating disease. One problem is that the cells must mature from the early fetal stage so they correspond to those in developed organs. New research – contradicting theories and textbooks – shows that specialized cells in fetal organs are not the only cells that can become stem cells. Under the right conditions, every cell can become a stem cell. This new knowledge simplifies the researchers' task of finding a formula for developing the cells.

By Morten Busch

What started out as a simple controlled experiment in the laboratory ended up requiring more than 5 years of hard work. Nevertheless, the results, which have recently been published in one of the world's leading journals, will require a comprehensive reformulation of the science textbooks used at universities. In addition, the results redefine the stem cell researcher's task of producing mature stem cells for such purposes as transplantation and therapy.

"Previously, the question was both which and how, but our new results show that we can now focus on how to induce cells to become exactly the type of stem cells we need. In addition, our study indicates that the body is able to use this: for example, in connection with severe injuries. This creates a new and unique starting-point for developing stem cell therapies and helping people who have injuries in the intestinal epithelium," explains Kim Jensen, Associate Professor, Biotech Research & Innovation Centre, University of Copenhagen.

Surprising plasticity

The researchers made this surprising discovery during their quest to understand how the fate of stem cells is organized in the intestine. Postdoctoral fellow Jordi Guiu tried to develop a method for following the fate of an individual cell in the intestine. He introduced fluorescent proteins into the intestinal cells to monitor the fate of an individual cell through many cell divisions by means of advanced microscopy.

"We were very surprised, because the literature stated that whether a cell could become a stem cell was predetermined. However, it turns out that all cells have the same ability to differentiate into a stem cell in the developed organ. This means that all cells in the fetal intestine can become stem cells if they receive the right signals."

"It is as if the cells are reset and lose some of the characteristics they have acquired in the developed organ."

Kim Jensen

Since the researchers could only explain a fraction of the intestinal growth from the cells previously thought to be fetal stem cells after their initial experiment, they collaborated with mathematics experts at the University of Cambridge. When they examined the data more closely, they surprisingly concluded that all cells in the intestine are equally able to develop into stem cells.

"To further test the surprising plasticity of the cells, we used a 3D culture system and transplanted the cells into mice. This confirmed our conclusion that all cells can become stem cells under the right conditions."

Reducing the challenges of stem cell therapy

The researchers observed another very interesting phenomenon about stem cells when they examined injured tissue in the intestinal epithelium (lining). In this situation, the body apparently induces cells in the immediate vicinity to transition into the primitive state that characterizes the cells in the fetal state.

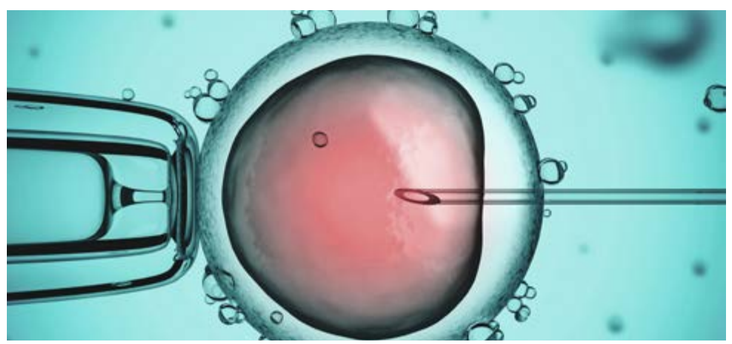
"It is as if the cells are reset and lose some of the characteristics they have acquired in the developed organ. Since we have found that many cells in addition to stem cells can participate in repairing tissue following injury, we are basing our work on the hypothesis that this is caused by fetal reprogramming."

Only a few treatment options currently use stem cells, and these stem cells are isolated from developed organs. One major challenge has been to find the fetal stem cells that are responsible for developing the organs.

"We can still only say with certainty that cells in the gastrointestinal tract have these characteristics. However, unpublished results indicate that this phenomenon is more common. In fact, we believe this is a general phenomenon throughout fetal development and for all cells."

If this turns out to be the case, it will reduce the challenges of stem cell therapy and repairing organs using stem cells from, for example, early fetuses.

"Alternatively, greater insight into the mechanisms by which the cells in the immature intestine mature to become stem cells could be used to better treat wounds that do not heal: for example, in the intestine."



The European Research Council, the H2020 research programme, Lundbeck foundation, Novo Nordisk Foundation, Carlsberg Foundation and the Marie Curie Fellowship programme and others supported this project. "Tracing the origin of adult intestinal stem cells" has been published in Nature by authors at BRIC and the Novo Nordisk Foundation Center for Stem Cell Biology (DanStem), University of Copenhagen.

How stem cells end up as fat cells or bone cells

New Danish research shows that stem cells deactivate many genes related to bone cells when they are on their way to becoming fat cells. This research is a masterpiece in basic science that can also eventually be used to improve stem cell therapy and understand various diseases.

By Kristian Sjøgren

Hundreds of genes must be activated or deactivated when stem cells become either bone cells or fat cells. This occurs in an organized and well-orchestrated network of mechanisms for regulating transcription, and Danish researchers have gained great insight into this process for the first time.

Transcription factors are proteins that bind to the DNA near the genes they regulate, and the research shows that many more changes in transcription factors are required to make a fat cell than to make a bone cell.

"We were surprised that stem cells, whether from bone marrow or from fatty tissue, resemble bone cells as much as they do. Many transcription factors involved in developing bone cells are already active in stem cells, so the stem cells are genetically preprogrammed to become bone cells. This means that these transcription factors must be deactivated and new fat cell–selective transcription factors activated before a stem cell can become a fat cell. Stem cells can form fat cells in several types of tissue. However, these stem cells appear to more closely resemble bone cells than fat cells," explains Susanne Mandrup, Professor, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense.

The new research results were recently published in *Nature Genetics*.

Can ultimately be developed into therapies to combat disease

This basic science discovery is important for understanding diseases in which stem cells lose their potential to repair and replace various types of tissue. Bones, for example, are constantly being formed and broken down, and failure of the processes that form the bones can lead to osteoporosis.

The discovery also adds knowledge that is vital for developing stem cell therapy to treat people with diseases in which the body lacks fat cells or bone cells. Doctors will hopefully be able to combat osteoporosis at some point by stimulating more stem cells to develop into bone cells. Doing this requires knowing how to optimally stimulate the process of maturing the stem cells.

"Most studies on how transcription factors influence the development of stem cells have examined individual transcription factors, but we have investigated the importance of all transcription factors and have thus created more comprehensive insight into how the process takes place. This is important for basic scientific insight into stem cell development but also provides knowledge that could be used to optimize stem cell therapy in various contexts," says Susanne Mandrup.

Developing stem cells into fat cells or bone cells involves 200 factors

All cells in the human body contain the same DNA and the same genes. What defines the properties of a cell is which genes in the cell can be transcribed and translated into proteins. For a stem cell to develop into a specific cell type, it must activate the specific genes characteristic of that cell type. Transcription factors govern this process.

Susanne Mandrup's research group investigated which transcription factors are involved in developing bone cells and fat cells. Advanced bioinformatic analysis revealed that, of the 933 transcription factors that appear to be involved in developing bone cells or fat cells, 202 transcription factors stimulate bone formation, and most of these are already active in the stem cell stage. Interestingly, these factors must be deactivated for a stem cell to develop into a fat cell, thus functioning as an on–off switch.

"We used a bioinformatic program we published last year that was developed by my talented postdoctoral colleague, Jesper Grud Skat Madsen. This program uses machine learning to identify which transcription factors play a role in regulating gene expression in a cell and during cellular development," explains Susanne Mandrup.

Overall structure of DNA determines whether genes are expressed

The researchers initially examined the changes in the structure of chromatin when stem cells become either fat cells or bone cells.

Chromatin is the protein scaffold in which DNA is organized in the nucleus, and this structure and the accessibility of the DNA determine which genes in DNA can be transcribed.

Depending on the chromatin structure, various transcription factors can access the relevant genes that direct a stem cell towards becoming either a fat cell or a bone cell.

"Here we discovered that bone cells have much in common with stem cells. Not just stem cells from the bone marrow but also stem cells from the fat tissue or muscles," says Susanne Mandrup.

Bioinformatics makes sense of the complexity

The researchers investigated whether they could use bioinformatics to predict which transcription factors the stem cells would activate or deactivate to become fat cells or bone cells.

More precisely, the researchers determined the position of the enhancers, which are the regulatory sequences in DNA where the transcription factors bind.

Some enhancers recruit transcription factors that activate genes related to bone cells, whereas others recruit transcription factors that activate genes related to fat cells.

"We have been able to predict which enhancers are important for the upregulation and downregulation of genes as the stem cells develop into bone cells or fat cells. Interestingly, some of these enhancers are already known based on genome variation studies to play a role in predisposing to various diseases," says Susanne Mandrup.

"Osteogenesis depends on commissioning of a network of stem cell transcription factors that act as repressors of adipogenesis" has been published in Nature Genetics. In 2018, the Novo Nordisk Foundation awarded a Challenge Programme grant of DKK 60 million to Susanne Mandrup, Professor, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense for the project ADIPOSIGN – Center for Adipocyte Signaling.

Immature cells zap around before settling down

Stem cells are already being used in combating previously untreatable diseases. Nevertheless, stem cells are not delivering their full potential because the production of specific cell types from stem cells cannot yet be managed. Researchers have now discovered the signals that determine the fate of immature cells in the pancreas. The research shows that they are very mobile and that their destiny is strongly influenced by their immediate environment. This breakthrough will facilitate the manufacturing of pancreatic islet cells for combating type 1 diabetes.

By Morten Busch

We are rapidly approaching the era for safe mass production of specialized neuronal cell types and insulin-producing beta cells. It will then be possible to test whether transplanting such cells will enable paralysed people to walk again or people with type 1 diabetes to restart their own production of insulin. Until now, the engineering of the specialized cells from pluripotent stem cells has largely been based on empirical knowledge of what works. Results published in the prominent journal Nature by a Danish-led research project represent a major leap forward.

"We have now been able to map the signal that determines whether pancreatic progenitor cells will become endocrine, such as insulin-producing beta cells or duct cells. The cells are analogous to pinballs, whose ultimate score is based on the sum of pin encounters. They are constantly moving around within the developing pancreas, leading to frequent environmental changes. We show that the exposure to specific extracellular matrix components determines the ultimate destiny of the cells," explains Henrik Semb, Professor and Executive Director, Novo Nordisk Foundation Center for Stem Cell Biology, DanStem, University of Copenhagen.

The matrix determines the destiny
Progenitor cells are similar to stem cells since they can both self-renew and differentiate into mature cell types. However, their self-renewal capacity is generally limited compared with that of stem cells. The dynamic behaviour of progenitors during organ formation makes them difficult to study. By seeding individual human stem cell–derived progenitors on micropatterned glass slides, the researchers could study how each progenitor, without the influence of neighbouring cells, reacts to its surroundings.

"This enabled us to discover something very surprising. Our investigation revealed that interactions with different extracellular matrix components change the mechanical force state within the progenitor. These forces result from interactions between the extracellular matrix, which is outside the cell, and the actin cytoskeleton, which is within the cell."

Pancreatic endocrine cells include all hormone-producing cells, such as insulin-producing beta cells and glucagon-producing alpha cells, within the islet of Langerhans, whereas the duct cells are epithelial cells that line the ducts of the pancreas.

"The experiments show that exposure to the extracellular matrix laminin instructs the progenitor cells towards an endocrine fate by reducing mechanical forces within the cells. Whereas exposure to fibronectin results in a duct fate because of increased mechanical forces."

Mechanism facilitates exploitation
To exploit their discovery, the researchers had to understand the signalling pathway. They showed that components in the extracellular matrix trigger a signal into the cell via an integrin receptor, resulting in changes in mechanical forces transmitted through the actin cytoskeleton. The yes-associated protein (YAP) then senses these forces to turn on and off specific genes.

"This cascade determines the ultimate fate of the progenitor cell. Perhaps the most astonishing achievement is that our data answer an enigma that has puzzled the field for decades. How some progenitors mature into duct cells, whereas others become endocrine cells via Notch signals."

The researcher show that the seemingly stochastic regulation of Notch function is in fact mediated by the progenitor's encounters with extracellular matrix interactions via the force-sensing gene regulator protein YAP. They were even able to validate the physiological relevance in vivo during pancreas development.

"We can now replace significant numbers of empirically derived substances, whose mode of action in current state-of-the-art differentiation protocols is largely unknown, with small molecule inhibitors that target specific components of the newly identified mechanosignalling pathway."

With this new strategy, insulin-producing beta cells can now be more cost-effectively and robustly produced from human pluripotent stem cells for future treatments against diabetes.

"Our discovery breaks new ground because it explains how multipotent progenitor cells mature into different cell types during organ formation. It also gives us the tools to recreate the processes in the laboratory, to more precisely engineer cells that are lost or damaged in severe diseases, such as type 1 diabetes and neurodegenerative diseases, for future cell replacement therapies."

"Mechanosignaling via integrins directs pancreatic progenitor fate decisions" has been published in Nature. Henrik Semb, Professor and Executive Director, Novo Nordisk Foundation Center for Stem Cell Biology, DanStem, University of Copenhagen, and head of Institute of Translational Stem Cell Research at Helmholtz Zentrum München is last author. Drs. Anant Mamidi, Assistant Professor, DanStem and Christy Prawiro DanStem share first authorship, and the work is the result of a collaboration with Professor Palle Serup's group, DanStem.. The Novo Nordisk Foundation has awarded grants of almost DKK 700 million (€92 million) to the Center for research between 2010 and 2018.

Under attack

Content

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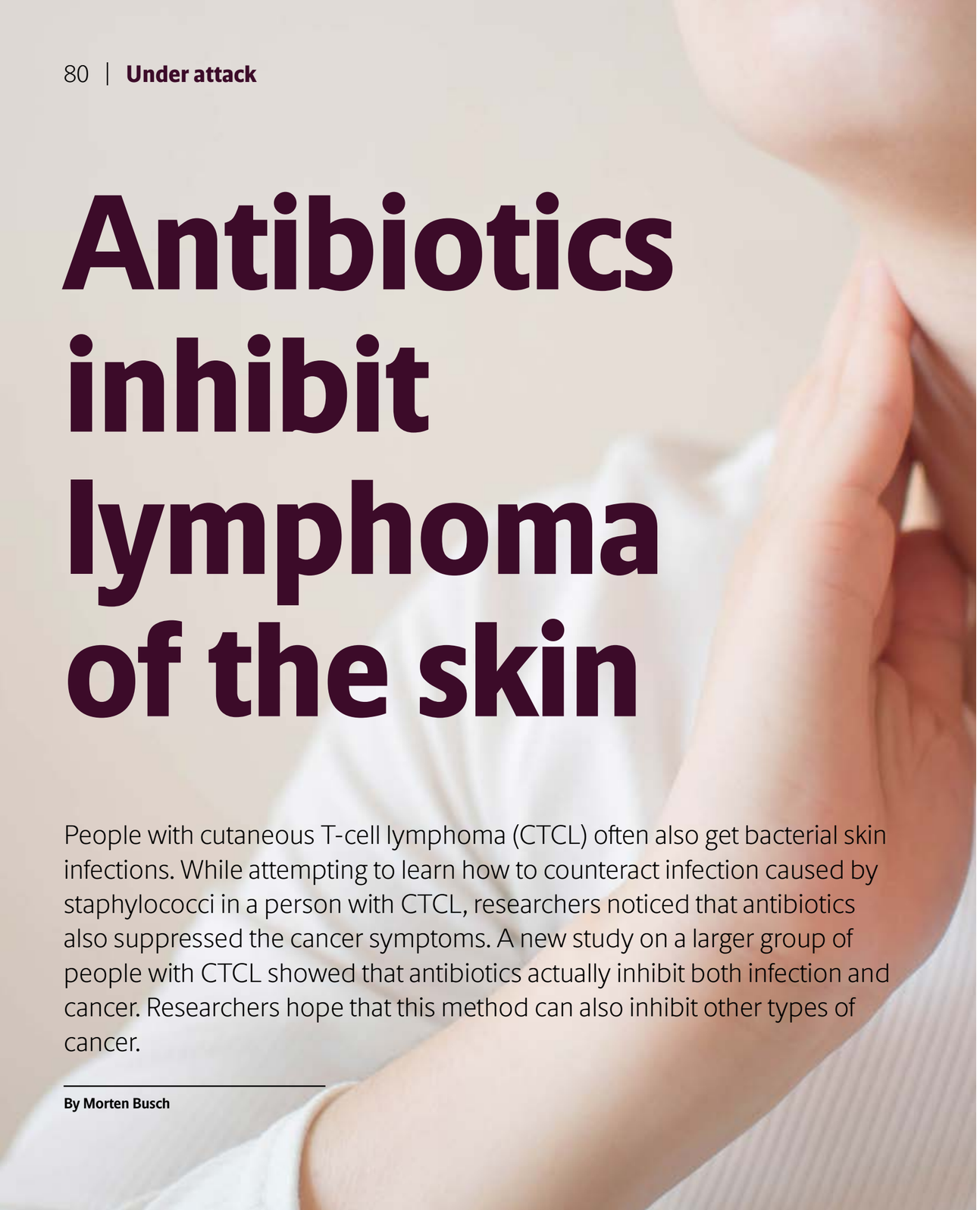
Researchers investigate how best to treat sepsis
In 2012, Danish researchers revolutionized the treatment of sepsis. Now they have begun another major clinical trial to investigate whether the way people with sepsis are most frequently treated is optimal.

Infections are associated with a greatly increased risk of epilepsy
An epileptic seizure can be a disturbing experience. Globally, about 50 million people have epilepsy, but only half of them know why. A new research project shows that the risk of developing epilepsy increases by 78% if a person has been hospitalized with an infectious disease. Additional hospitalizations for infection increase the risk further.

New research: People with high blood pressure should get a flu shot every year
It is well known that the flu can be dangerous for older and frail people. However, new research shows that the flu is more dangerous for people with high blood pressure. A large population study among people with high blood pressure shows that flu vaccination is associated with a reduced risk of death. The researchers behind the study think that people with high blood pressure should get a flu shot every year.

Major study links parasite in cat excrement to schizophrenia
A large-scale study has linked infection with the *Toxoplasma* gondii parasite (toxoplasmosis) to an increased risk of developing schizophrenia. The study is the first to examine the relationship between when people develop toxoplasmosis and when they develop schizophrenia.



Antibiotics inhibit lymphoma of the skin

People with cutaneous T-cell lymphoma (CTCL) often also get bacterial skin infections. While attempting to learn how to counteract infection caused by staphylococci in a person with CTCL, researchers noticed that antibiotics also suppressed the cancer symptoms. A new study on a larger group of people with CTCL showed that antibiotics actually inhibit both infection and cancer. Researchers hope that this method can also inhibit other types of cancer.

By Morten Busch

”We were positively surprised to discover that the staphylococci in the skin secrete toxins that cause immune cells to release cytokines, which are important signalling substances.”

Lars Iversen

When people develop cancer, the cancer cells and the body's other cells begin to battle over resources. The outcome of this conflict is crucial. If the cancer cells receive the necessary nutrition, they grow and divide and the cancer spreads. Researchers are therefore constantly trying to find new methods to starve cancer cells without harming the body's other cells. One surprising method of achieving this arose unexpectedly.

"When people develop CTCL, the immune system's T cells develop abnormally and attack the skin. People with CTCL are often also plagued by bacterial skin infections. We attempted to counteract these bacteria with antibiotics and discovered that they also inhibited the cancer. We now know the reason: the toxins the bacteria produce stimulate the growth of the cancer cells. By removing the bacteria, we remove the extra fuel the bacterial infection pours on the cancer fire," says Niels Ødum, Professor, LEO Foundation Skin Immunology Research Center, University of Copenhagen.

Bacteria boost the growth of cancer cells

CTCL is a rare type of non-Hodgkin lymphoma that affects the skin. In the vast majority of people with non-Hodgkin lymphoma, it affects the B cells (memory cells) of the immune system. However, among people with the less common CTCL, it affects the T cells. These cells contain the immune system's key defence system, tasked with finding cells in the body that have been infected by either viruses or bacteria.

"In the past, doctors were very reluctant to prescribe antibiotics to people with skin infections because they feared that antibiotic-resistant bacteria would develop. Our new research changes that view. We got the idea from laboratory tests showing that the toxins the bacteria produce can boost cancer cell growth in test tubes."

Lars Iversen, Clinical Professor, Department of Dermatology and Venereology, Aarhus University had also noticed that prescribing antibiotics for blood poisoning noticeably improved the skin of a person with CTCL. The researchers' idea, therefore, was to give people a very powerful antibiotic regimen that could effectively combat staphylococcal infection to prevent it from recurring. The researchers also investigated how this affected the cancer cells.

"We were positively surprised to discover that the staphylococci in the skin secrete toxins that cause immune cells to release cytokines, which are important signalling substances that give the cancer cells an extra boost, enabling them to grow and divide more easily. By eliminating the staphylococci, we removed that boost so the cancer cells lost their growth advantage over the body's other cells. This treatment was like removing a lot of fuel from the fire."

The new antibiotic treatment has only been tested on eight people, but it reduced the fraction of malignant T cells considerably among six of these people. All participants experienced a marked decrease in clinical symptoms in response to this aggressive, short-term treatment with antibiotics.

"Because of the fear of antimicrobial resistance, we were excited to see whether the effect would last, but we still found improvements for up to 6 months after the treatment ended. Antibiotic treatment therefore appears to break a vicious circle and slow the increasingly rapid growth of cancer cells."

Alternative to current cancer treatment

The new results are pioneering in many ways. Antibiotics have previously been reported to improve the symptoms of some individuals, but researchers had never previously investigated how antibiotics affect cancer.

"The results are groundbreaking because this is the first time that bacteria and CTCL have been directly linked. The research is the result of many years of work combining molecular and genetic studies in laboratory experiments with clinical studies of carefully selected people."

However, there is a way to go between pilot experiments involving eight people and achieving a standard treatment. Before this can happen, major clinical trials need to be carried out to determine the people and the disease stages that are optimal for this treatment. Nevertheless, the researchers are so positive that they are already investigating whether the treatment can be used in other contexts.

"We think that the same type of treatment could inhibit other types of skin cancer. We are now investigating how to reinforce the current cancer treatment with antibiotics and to find new ways to attack the bacteria without using antibiotics. In other words, finding new agents that can remove the growth benefits that bacteria give the cancer cells in their struggle against the body's immune system – without risking the development of antimicrobial resistance."

"Antibiotics inhibit tumor and disease activity in cutaneous T cell lymphoma" has been published in Blood (Journal of the American Society of Hematology). The study is a collaboration between the LEO Foundation Skin Immunology Research Center, University of Copenhagen; Aarhus University and Aarhus University Hospital; Zealand University Hospital and collaborators in the United States and Germany. The study received support from the LEO Foundation, the Novo Nordisk Foundation (Tandem Programme grant), Independent Research Fund Denmark, the Lundbeck Foundation, the Danish Cancer Society and TV2's Beat Cancer Fundraiser.

Gigantic virus gives researchers a headache

Researchers have identified a giant virus that attacks a type of bacteria known to be associated with the development of diabetes and obesity.

By Kristian Sjøgren

A gigantic virus is giving researchers a headache – not because they are being infected with some exotic disease but because they cannot understand how some viruses get to be so large.

The cross-assembly phage (crAssphage) is a phage – a virus that infects bacteria – that is 10 times larger than HIV and is present in the intestines of half the world's population. CrAssphage does not attack humans but attacks bacteria of the genus *Bacteroides*.

Bacteroides species are known to be involved in developing diabetes and obesity, so researchers are very clearly interested in understanding these gigantic viruses.

Now researchers have sequenced the entire genome of crAssphage.

"These viruses are so large that researchers are beginning to wonder whether they are viruses or whether they are something else. And then it is interesting that they have the potential to affect our health because they live by infecting bacteria, which are linked to many human diseases," explains Frank Møller Aarestrup, Professor and Head, Research Group for Genomic Epidemiology, National Food Institute, Technical University of Denmark, Lyngby.

The new study has been published in *Nature Microbiology*.

Megaphages can be larger than some bacteria Phages and megaphages are not normally considered to be living organisms. Instead, they are classified as large molecules with a genome in the form of RNA or DNA.

When phages infect bacteria, they can either assume the function of the bacteria or cause the bacteria's cellular mechanisms to copy the phage's DNA and RNA until the bacteria rupture and new phages flow into the surroundings.

However, megaphages give researchers a headache. Although most phages are much smaller than the bacteria they infect, the Lak megaphage (with Frank Møller Aarestrup also participating in its recent discovery) is larger than the smallest bacteria and thus difficult to classify as anything other than a living organism.

More phages than bacteria

Bacteria of the Prevotella genus are thought to be the hosts of the Lak phage, and *Bacteroides* for crAssphage. The dominance of *Bacteroides* in the gut is generally associated with negative health effects among humans, whereas Prevotella is linked to positive effects.

Prevotella helps people digest stubborn plant material and promotes health.

"For example, when we analyse the total DNA in a stool sample from a pig, up to 2% is from the Lak phage. This is a large amount of DNA and almost as much as the amount of Prevotella DNA. This is sort of like having almost as many lions as wildebeest on the savannah, which makes no sense. All this indicates that that we do not yet fully understand these megaphages. Maybe they have other hosts, or their biology differs from what we think it is," explains Frank Møller Aarestrup.

Phages have coevolved with primates for millions of years

The researchers collected stool samples from around the world and analysed the DNA in them. By studying the relationship between the concentrations of specific parts of the genome in the numerous samples, the researchers can pool the genetic materials into groups that comprise whole genomes.

"These viruses are so large that researchers are beginning to wonder whether they are viruses or whether they are something else."

Frank Møller Aarestrup

The researchers used this method to identify the huge crAssphage genome and subsequently prove in the laboratory that it was really just one type of phage.

The researchers found the phage in more than half the samples they analysed. The sequences came from more than one third of the world's countries and from all continents.

In addition, researchers have also found crAssphage-like sequences among primates, suggesting that the guts of humans and other primates have been home to these phages for millions of years.

The strong association with humans can be an inherent headache for researchers.

"CrAssphage appears to be more associated with people than with *Bacteroides*. CrAssphage has coevolved closely with humans, similarly to herpesvirus and to bacteria of the Helicobacter genus. However, the phage does not infect us, which makes the association unexplained. It is tempting to think that crAssphage does not infect *Bacteroides* but infects something else and is transmitted from mother to child, but many unanswered questions remain, and we do not yet understand this," says Frank Møller Aarestrup.

Using phages to cure diseases and maintain a healthy gut

The researchers have learned, however, that both the Lak phage and crAssphage are megaphages that strongly influence the composition of bacteria in the gut.

The composition of bacteria in the gut is directly linked to numerous diseases: autism, Alzheimer's, obesity, diabetes and intestinal diseases such as Crohn's disease and ulcerative colitis.

These huge phages are therefore potentially interesting pharmaceutically if medicine or genetic engineering can get them to behave differently to alter the composition of gut bacteria in one direction or another. This is called phage therapy.

For example, perhaps improving the ability of crAssphage to infect *Bacteroides* could reduce the bacterial risk of developing obesity or diabetes.

Perhaps crAssphage, Lak phage and other megaphages could create a balance in the composition of gut bacteria that would enable the whole gut environment to better counteract pathogenic bacteria such as *Salmonella* species.

"We are still in the early stages of this research, trying to discover and identify the megaphages. The next natural step is to study their function and what they do in the real world. For starters, we just need to simply determine whether they really infect the bacteria we think they infect and whether they might also infect other bacteria that may affect people's health," says Frank Møller Aarestrup.

"Global phylogeography and ancient evolution of the widespread human gut virus crAssphage" has been published in Nature Microbiology. In 2016, the Novo Nordisk Foundation awarded a Challenge Programme grant of DKK 60 million to Frank Møller Aarestrup for the project Global Surveillance of Antimicrobial Resistance.

Combating antimicrobial resistance in global sewers

A Danish research project suggests that poverty and poor sanitary conditions may be a more important cause of the global problem of antimicrobial resistance than the excessive use of antibiotics.

By Kristian Sjøgren

The prevalence of antimicrobial resistance has never been higher and more relevant than in 2019. More multidrug-resistant bacteria are now lurking in the world's hospitals than ever before, and worldwide, researchers are trying to find the next new antibiotic that can save the lives and limbs of people when everything else fails.

But perhaps the research world is approaching the problem incorrectly. At least, this is what a Danish researcher believes: that the battle against multidrug-resistant bacteria has become focused in the wrong direction.

"For many years, we have focused on the last part of the long chain that leads to the problem: the multidrug-resistant bacteria among critically ill people in hospitals. These are the people we are trying to help by developing new types of antibiotics, rapid diagnostic tests or infection control. Afterwards, we try to patch the system when things go wrong, instead of preventing and thereby ensuring that the problems do not arise. If we can instead reduce the general transmission of infection and ensure that antibiotics work the first time, we will not need to develop new antibiotics at all, because then we would not have any antimicrobial resistance," explains Frank Møller Aarestrup, Professor, National Food Institute and Head, Research Group for Genomic Epidemiology, Technical University of Denmark, Lyngby.

In 2016, the Novo Nordisk Foundation awarded a Challenge Programme grant of DKK 60 million to Frank Møller Aarestrup to test his theories that the problem of antimicrobial resistance can be examined differently. The grant runs until 2023.

Focusing on far too few types of bacteria

Frank Møller Aarestrup believes that society, including the research community, has been incorrectly focusing for a long time on developing weapons to combat the transmission of antimicrobial resistance.

Globally, only 5–10% of antibiotics is used in hospitals, yet almost the entire focus is on individual cases of antimicrobial resistance that emerge in individual hospital locations. This could include multidrug-resistant gonorrhoea in England or vancomycin-resistant enterococci in Copenhagen.

Denmark, for example, focuses enormously on methicillin-resistant Streptococcus aureus (MRSA), with each of the few cases of MRSA being documented and analysed in minute detail. However, MRSA only represent about 0.5% of all cases of staphylococcal infection, and staphylococci only account for a fraction of all cases of bacterial infection.

Further, many people die from infectious diseases caused by non-resistant bacteria, but there is not much public focus on this.

According to Frank Møller Aarestrup, the problem is that the concept of resistance has taken on a life of its own in the public debate. There is a disconnect between the discussion and the actual problem, which is the transmission of infectious diseases.

"One of our collaboration partners has developed advanced computer models that show where to focus to minimize the number of deaths from infectious diseases. The models show very clearly that trying to control the transmission of infection and thus the development of resistance has a much greater effect in minimizing the total number of deaths than focusing on rare problems in hospitals. Unfortunately, the political focus is primarily on the rare cases of multidrug-resistant bacteria, which is probably because improving hygiene and the like, which we know really works, is not as sexy as the technological solutions of developing new types of antibiotics," says Frank Møller Aarestrup.

We do not know where resistance develops

According to Frank Møller Aarestrup, solely focusing on the last part of the chain that leads to antimicrobial resistance also results in missed opportunities to intervene earlier to prevent antimicrobial resistance from occurring.

An example of this is where antimicrobial resistance is almost always discovered: in high-income countries, mainly Europe and the eastern United States. For example, MRSA was discovered in the United Kingdom.

Does this mean that the resistance problem in this case originated in the United Kingdom?

Probably not, but it is unclear where MRSA actually originated, because researchers first focus on the problem after it arrives in high-income countries: when multidrug-resistant bacteria are discovered in a hospital.

Nevertheless, the general consensus among researchers is that antimicrobial resistance most likely originates in Asia and that people on holiday in Asia get infected and bring the multidrug-resistant microbes home.

However, not everyone, including Frank Møller Aarestrup, agrees with this assumption.

"The problem is that we have not actually investigated whether this hypothesis is correct. We assume that, because Asia supposedly uses more broad-spectrum antibiotics than Europe, North America and Oceania, bacteria can more easily develop antimicrobial resistance there. However, we do not know whether this is true because we have not investigated this," he says.

Antimicrobial resistance is exploding in Africa

In fact, Frank Møller Aarestrup's research shows that many assumptions on the origins of the development of antimicrobial resistance are simply wrong.

As the first stage of this major research project based on a grant from the Novo Nordisk Foundation, Frank Møller Aarestrup and his research team from the Technical University of Denmark carried out a pilot study that took samples from sewage treatment plants in 60 countries and investigated the concentration of multidrug-resistant bacteria.

The results showed that China and India did not have the highest prevalence of antimicrobial resistance, as researchers in Europe and North

America have always believed. Africa had a high prevalence of antimicrobial resistance, despite limited access to antibiotics in many parts of Africa.

According to Frank Møller Aarestrup, the difference between the assumptions of the research community and the new observations may be that China and India have far more researchers and publish much more scientific literature on the subject than the other Asian countries. These researchers actually write more about the prevalence and transmission of antimicrobial resistance in China and India, but this does not necessarily reflect the real picture.

"These initial results also surprised us because they contradicted what many other researchers thought. I was also very surprised that Africa has so much antimicrobial resistance and that we may be wrong about China and India," says Frank Møller Aarestrup.

Investigated sewage in 60 countries
The researchers simply collected samples from wastewater treatment plants in 60 countries and sent them to Denmark, where researchers extracted all the DNA. They then sequenced uniform fragments of the DNA and matched it against a database of gene sequences for known antimicrobial resistance genes.

This gave the researchers an overview of the number of multidrug-resistant bacteria in a given sewage sample and insight into which antibiotic resistance genes they have.

Taking sewage samples has several advantages.

- Getting stool samples from a random population sample is very difficult because of concerns about privacy, including personal data protection. However, once the stool has been flushed down the toilet, it is no longer connected to the person and becomes an environmental sample that researchers can scrutinize fully.

- Second, it is much easier to take a sample from a wastewater treatment plant, in which bacteria from thousands of people are already mixed together, to get a representative sample of the prevalence of antimicrobial resistance in an often large catchment area that empties its toilets into the same sewerage system.

"We work very closely with the World Health Organization (WHO) because it gives us both legitimacy and access to healthcare systems around the world."

Frank Møller Aarestrup

"We work very closely with the World Health Organization (WHO) because it gives us both legitimacy and access to healthcare systems around the world. This makes our research much easier," says Frank Møller Aarestrup.

The computer for analysing the data is being developed
Performing this type of analysis may sound very simple, but it is not. Analysing and comparing the quantity of data in the various databases and in the sewage samples from the researchers' next project requires the total capacity of Denmark's most advanced supercomputer, Computerome, for a whole month.

In fact, the computer has not been completely finished and will not be ready until early summer 2019. Frank Møller Aarestrup has been given exclusive rights to use the computer in the first month to analyse his data.

If he could not use this computer, analysing each sample would have taken about 22 days, and in the follow-up research, which aims to confirm the results of the pilot project, he has 268 samples from 103 countries. Figure out how long that would take?

Two global groups in the prevalence of resistance
The researchers have already obtained quite interesting results from the pilot project.

First, the prevalence of antimicrobial resistance varies considerably by region.

Africa, Asia and South America are one group, and North America, Europe and Oceania (Australia and New Zealand) are the other. Antimicrobial resistance is very uniformly distributed in South America, Asia and Africa, which means that the bacteria have developed resistance uniformly to all types of antibiotics, whereas in North America, Europe and Oceania, the bacteria are mainly resistant to macrolide antibiotics.

Some places deviate, however. These include the Galápagos Islands, with a resistance profile similar to that in Europe, North America and Oceania, and Malta, with a profile similar to that in Africa.

"The explanations for this may be that many tourists from high-income countries visit the Galápagos Islands and that Malta is relatively close to Africa," says Frank Møller Aarestrup.

The study was published in Nature Communications.

Tenuous link between use of antibiotics and the prevalence of antimicrobial resistance
In analysing the results, the researchers from the Technical University of Denmark tried to explain the findings for the prevalence and distribution of antimicrobial resistance.

One thing they examined is whether the total use of antibiotics in a country is associated with the prevalence of antimicrobial resistance. They also investigated whether antibiotic resistance genes in sewage samples can be linked to people travelling between two specific countries. Thus, is the prevalence of specific antibiotic resistance genes higher among countries that have many direct flights between them?

- The researchers found no association between antimicrobial resistance and air travel, which is one of the main arguments for the dominant theory of how antimicrobial resistance is transmitted from Asia to the Western Hemisphere.

- Even more interesting perhaps is that the researchers found the prevalence of antimicrobial resistance is very weakly associated with the use of antibiotics in countries.

"We really had to struggle to find any association. It was very weak even though the entire research world suggests that this pathway is the villain in developing antimicrobial resistance. This is rather worrying that the association that everyone is certain exists is so difficult to find. This can keep researchers awake at night, including me," explains Frank Møller Aarestrup.

Poor sanitary conditions may enable antimicrobial resistance to be transmitted
To find another explanation for how antimicrobial resistance develops, Frank Møller Aarestrup used the Human Development Index of the United Nations Development Programme, which indicates the state of development of countries. It is based on World Bank data, which describes the infrastructure investment, wage levels and investment in sewage and healthcare for each country.

"It is probably the closest we can get to the original data from and situation of individual countries," says Frank Møller Aarestrup.

When the researchers linked the prevalence of antimicrobial resistance with the Human Development Index in various countries, they found a very clear association that was much stronger than the association with the use of antibiotics.

The researchers subsequently extracted 1503 subcomponents of the World Bank data and linked them directly with the prevalence of antimicrobial resistance. They found very strong associations with investment in hospital services and investment in sewerage systems, but none for such parameters as the number of copper mines, forested areas and the like.

"This showed very clearly that investment in sanitary conditions is much more strongly associated with the development of antimicrobial resistance than the use of antibiotics," explains Frank Møller Aarestrup.

Antibiotics in high-income countries disappear into the toilet
According to Frank Møller Aarestrup, it makes sense that sanitary conditions are a much stronger cause of antimicrobial resistance than antibiotic use.

The problem of antimicrobial resistance arises only when multidrug-resistant bacteria are transmitted. In a low-income country where many people still have to defecate on the streets, bacteria with newly acquired antimicrobial resistance can much more easily be transmitted, infect other people and become a problem than in a high-income country such as Denmark, for example, where antimicrobial resistance has greater difficulty in being transmitted because of higher hygiene standards and better sanitary conditions.

In almost all cases, multidrug-resistant bacteria are flushed down the toilet, never to be seen again.

"The World Bank is very pleased with the result and therefore now has an investment objective of slowing down the transmission of multidrug-resistant bacteria. Investing in restricting the use of antibiotics is difficult, but improving sanitary conditions in low-income countries is easier to invest in," says Frank Møller Aarestrup.

Monitoring pathogenic bacteria in real time
In addition to studying sewage samples from various parts of the world, Frank Møller Aarestrup is launching several projects aimed at supporting these new and, for some, controversial conclusions.

- One project the researchers have created will examine the development of resistance over time from one wastewater treatment plant. The researchers will then investigate whether this is associated with the findings from hospitals in the region during the same period: that is, whether the overall development of resistance in society is associated with the specific problems of hospitals.

- Second, the researchers will also collect samples from socioeconomically diverse places in the same country, including in the United States. Here the researchers will determine whether the prevalence of antimicrobial resistance is higher in areas with low income than in areas with high income and thereby confirm the findings from the comparison with data from the World Bank.

"This is linked with another project that stemmed from the project funded by the Novo Nordisk Foundation. Here we will take samples from 14 locations in the United States and 10 African countries. Ideally we would have liked to carry out a study to determine the effect of implementing better sanitary conditions, but it is not easy to take samples from a country, ask them to invest DKK 3 billion in better sanitary infrastructure and then take samples again. This is the second best option," says Frank Møller Aarestrup.

WHO may take over the project
Linked to the whole project of discovering the primary source of development of resistance is a project in which the researchers investigate the prevalence of various pathogenic bacteria in sewage samples.

Here, the researchers want to identify the prevalence of cholera bacteria, *Salmonella*, *Campylobacter* and others so that they can monitor the disease situation in a specific area in real time.

For example, if the number of cholera bacteria increases in sewage samples separated by 2 weeks, this indicates that an outbreak may be on the way, and through appropriate measures, public health authorities can mitigate the severity of the outbreak.

"The purpose of the projects is that we can transfer them to others in 5 years, so that a large international organization such as WHO can monitor the prevalence of bacteria and antimicrobial resistance in many countries. This will give use a much better overview of the factors involved when antimicrobial resistance develops, and we can put this into a general context of disease development and thus make data much more relevant to the population – and not only hospitals and researchers," explains Frank Møller Aarestrup.

"Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage" has been published in Nature Communications. In 2016, the Novo Nordisk Foundation awarded a Challenge Programme grant of DKK 60 million to Frank Møller Aarestrup for the project Global Surveillance of Antimicrobial Resistance.

Smuggling route: **How the body defeats DNA parasites**

People and all other organisms are constantly involved in an arms race against genetic parasites such as retroviruses and transposons. Research on fruit flies now reveals how host cells make copies of the DNA parasites and smuggle these copies into the genome-defence system. This new knowledge can be used to understand the human genome-defence system and can help researchers learn how to correct malfunctions.

By Morten Busch

The human genome comprises 3 billion DNA building blocks, of which fewer than 2% are protein-coding genes. Of the remainder, about two thirds are genetic parasites such as retroviruses and transposable elements (transposons) and fragments thereof. In short, our genome is a genetic battlefield that is constantly being invaded by genetic parasites, which are then silenced by the genome-defence system.

"We have now identified some parts of how host cells counter-attack in this evolutionary battle. In 2017, we found the moonshiner gene and its Moonshiner protein in fruit flies that produce the illegal copies. Now we have discovered the bootlegger gene and its Bootlegger protein that disguise the transport of the small copies into the cell. By understanding these mechanisms in model organisms, we can build a knowledge framework that enables us to explore this biology in humans and thereby build a foundation for rectifying aberrations in the genome-defence system," explains Peter Refsing Andersen, Assistant Professor, Department of Molecular Biology and Genetics, Aarhus University.

Dissecting ovaries from fruit flies

This molecular arms race occurs in virtually all forms of life. A species can die out as a result of massive damage to its DNA if its genome-defence system fails to control the genetic parasites. The host cells therefore have to silence the copying of the genomic parasites. Nevertheless, they need to recognize the genetic sequence of the parasites so they can differentiate between the parasitic genes and the host cell's normal genes.

"The main weapon of genetic parasites is getting their host to copy their genome. Host cells therefore attempt to silence the copying process through the genome-defence system, which protects their DNA against the uncontrolled proliferation of transposons. However, the genome-defence system must use small copies of these DNA parasites to enable the cell to recognize them."

In 2017, Peter Refsing Andersen and his colleagues published the first landmark finding of the moonshiner gene in Nature. The new discovery is equally spectacular and has been published in Cell.

"In 2017, we discovered an alternative copying mechanism that permits the cell to make small RNA copies of the DNA from the genetic parasites. We have now identified the transport mechanism the cell uses to smuggle the small copies out of the nucleus past its own defence system: a smuggling pathway. This method has many similarities with how HIV evades a host's defence system."

The researchers found the new genes and their proteins in the reproductive cells of fruit flies. The reason they searched there is because that is where the genetic parasites attempt to replicate themselves so that they can propagate their new copies to the next generations. It is therefore remarkable that these defence systems have only been found there.

"These experiments are relatively simple in fruit flies, because the ovaries comprise one third of the bodies of females. We followed their journey through the cell by labelling the small RNA copies with fluorescent substances in the ovaries' reproductive cells. Combined with the analysis of RNA and protein from dissected fruit fly ovaries, we have uncovered the entire molecular pathway that exports the RNA copies of the genetic parasites from the cell nucleus to the cytoplasm, where the genome defence is loaded with the small RNAs that guide it to the parasitic genes."

Surprisingly rapid development

After the researchers discovered the smuggling pathway, they tried to remove this genetically in the fruit flies. The fruit flies' reproductive cells became sterile as a result because they had been conquered by the genetic parasites. The researchers use fruit flies as a model because their genome is similar to that of humans, but it is still too early to determine whether the human system is identical.

"We use fruit flies to understand the molecular mechanisms of genetic activity, and although human genes are not completely identical, we have already found the genes that are key in the mechanisms of fruit flies in mice and humans. Since these genes are so similar, we can almost certainly say that the genes exist and that the concepts are conserved in humans."

Peter Refsing Andersen very much looks forward to discovering even more similarities, as more and more people are genetically sequenced in the coming years. This is the only way we can become much wiser about how the human system functions and the significance of errors in the genome-defence system.

"We also need to learn how to correct these errors when they occur. Our results mainly testify to the constant and rapidly evolving arms race between host genomes, such as the human and its genetic parasites. It is extremely fascinating to discover how we decode each other's mechanisms and how we have to bypass our own security systems through hacking and smuggling to win the war."

"A heterochromatin-specific RNA export pathway facilitates piRNA production" has been published in Cell. In 2014, the Novo Nordisk Foundation awarded a postdoctoral fellowship to co-author Peter Refsing Andersen to carry out research abroad on the project Breaking Down the Rules of Transcription in Defence of the Genome. The postdoctoral fellowship involved a 4-year study period mainly at the Institute of Molecular Biotechnology of the Austrian Academy of Sciences, Vienna. He was recently awarded a Hallas-Møller Emerging Investigator grant of DKK 10 million to establish his own research group at the Department of Molecular Biology and Genetics, Aarhus University to study genetic parasites. Read more about the on-going research in the laboratory of Peter Refsing Andersen here.

Unexpected chain reaction leads to mass destruction

The human intestinal system is a battlefield between the "good" bacteria that, for example, help with digestion and the "bad" bacteria that can make us sick. One weapon the body uses to kill the bad bacteria is antimicrobial peptides, which are small protein fragments. However, the body produces enzymes that split the antimicrobial peptides into hundreds of even smaller fragments. Researchers had thought that this process destroyed the weapon, but new research shows that each small fragment specifically attacks and kills especially bad bacteria. The researchers will now develop this naturally occurring fragmentation bomb to provide an alternative to antibiotics and to improve general intestinal health.

By Morten Busch

"These results significantly expand our understanding of how the body maintains balance in our microbiome and how complex the intestinal defence mechanisms are."

Benjamin Anderschou Holbech Jensen

The task sounds simple enough: fight the enemy and support the allies. But understanding these processes can be difficult when this struggle takes place in the innermost part of our intestinal system. Nevertheless, understanding the defence mechanism in people's gut is extremely important because it is key to avoiding disease and strengthening health. Now researchers have made a major breakthrough in understanding how the intestinal system keeps external enemies from invading the body.

"The Paneth cells that line the small intestine secrete defensins: small protein fragments that can kill pathogenic bacteria. We did not understand why the intestinal fluid itself seems to split some of these defensins into hundreds of smaller fragments. Now we can see that this process does actually does not destroy the defensins; on the contrary, it creates hundreds of new and more specific weapons. Now we hope to copy this fragmentation bomb to treat bacterial infections – as an alternative to the antibiotics we have today that are increasingly ineffective," explains a researcher behind the study, Benjamin Anderschou Holbech Jensen, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Skewed bacterial balance

To understand why the defensive weapons secreted by the Paneth cells of the small intestine were apparently neutralized as soon as they entered the intestine, the researchers decided to examine how the enzymes –proteases – in the duodenal fluid of the small intestine would react in the laboratory with two of the most important small intestinal defensins: human α-defensin 5 (HD-5) and 6 (HD-6).

"We used liquid chromatography and mass spectrometry to monitor how the enzymes fragmented the defensins. This enabled us to both identify each defensin fragment and test how they affected the growth of different types of bacteria. The proteases apparently did not affect HD-6 at all due to the formation of a protective peptide nanonet shielding this particular defensin from the enzymatic degradation, whereas HD-5 was split into hundreds of small fragment combinations. The scientific community at large, expected that this would inactivate its antimicrobial properties. So we were surprised when we tested how the fragments affected different bacteria."

The researchers had expected that the HD-5 fragments would be wholly or partly ineffective in retarding the growth of bacteria. Instead, the hundreds of fragments produced new and even more active combinations, and the researchers found that HD-5 positively affected the bacterial balance.

"The pathogens died off, and even more favourable conditions were created for the bacteria that are desirable in the gut. So a single simple peptide, HD-5, functions as a kind of fragmentation bomb that seems to be key in fine-tuning our intestinal system and providing a healthy balance."

Promising pathways for developing drugs

The new finding is surprising because researchers had thought that biological degradation inactivates one of the intestine's most important weapons against external enemies. Instead, the degradation process leads to hundreds of fragments, each of which has unique specificity and a strategy for eliminating bacteria.

"We sensed that HD-5 in particular and defensins in general must be important, since evolution has preserved them for millennia and across different species, including plants, fish, birds and mammals – with very little variation between species. They are thus one of the most frequently conserved defence mechanisms in biology. We are extremely fascinated that evolution has actually developed clusters of fragments that are further activated when they reach the intestine and whose cocktail is so targeted and effective against bad bacteria – without damaging the good bacteria."

This discovery may prove very valuable in fighting the multidrug-resistant bacteria that existing antibiotics cannot eliminate. The really good news is that, although HD-5 is a relatively long protein fragment of 34 amino acids that strongly depends on an extremely specific structure that is difficult to produce synthetically, producing the typical nine-peptide-long linear microfragments that HD-5 becomes when split would be relatively simple.

"These results significantly expand our understanding of how the body maintains balance in our microbiome and how complex the intestinal defence mechanisms are. They also show extremely promising avenues for developing drugs to combat not only bacterial infections but also diabetes, cardiovascular diseases and other complications related to the microbiome since our other experiments show that the defensins significantly lower cholesterol levels and, at least in mice, can reduce the risk of developing nonalcoholic fatty liver disease and also treat it. We are currently expanding these studies to specific human models of diabetes-accelerated arteriosclerosis and general atherosclerosis, which cause nearly one third of all deaths in high-income countries."

"Paneth cell α-defensins HD-5 and HD-6 display differential degradation into active antimicrobial fragments" has been published in Proceedings of the National Academy of Sciences of the United States of America. "Human Paneth cell α-defensin 5 treatment reverses dyslipidemia and improves glucoregulatory capacity in diet-induced obese mice" has been published in the American Journal of Physiology, Endocrinology and Metabolism. In 2017, the Novo Nordisk Foundation awarded a grant to Benjamin Anderschou Holbech Jensen for the project A Novel Oral Combination Therapy Targeting the Gut Microbiome to Alleviate Insulin Resistance and Type 2 Diabetes–linked Aortic Valve Stenosis.

Half the children in Guinea-Bissau have intestinal parasites

The researchers set out to study the immune system of children with intestinal worms in Guinea-Bissau. However, they were surprised when they investigated the samples from sick children. Fewer children than expected had intestinal worms, but half had intestinal parasites. Most surprisingly, the researchers also found that the same percentage of healthy children had the parasites. The researchers now aim to identify which children have harmful parasites and which children have parasites that may even be beneficial.

By Morten Busch

Guinea-Bissau is one of the poorest countries in the world, and this affects health: 55 of 1000 children die at birth, people with diabetes are rarely diagnosed and treated, and cancer is simply a death sentence. Sewerage and sanitation are also incredibly poor, making infections an everyday event. Researchers have now investigated one of the worst plagues affecting the country's children – intestinal parasites that lead to malnutrition, impaired quality of life and cognitive problems.

"Our goal was to investigate the immune system of children with intestinal worms so we can diagnose and treat them. Fortunately, treatment efforts in recent years appear to have significantly reduced the proliferation of these worms. Nevertheless, half the children had intestinal protozoa. We will now develop a method to identify the children who need treatment and the children to whom the parasites are harmless or even beneficial," explains a main author, Sebastian Leicht von Huth, Visiting Researcher, Department of Cancer and Inflammation Research, University of Southern Denmark, Odense.

A big surprise

The research is part of the Bandim Health Project that has operated since the late 1970s, in which researchers from Statens Serum Institut and various universities in Denmark monitor more than 200,000 people in urban and rural areas of Guinea-Bissau, including the effects of vaccines, vitamin supplements and other measures to improve health.

"The initial plan was to examine some of the signalling molecules in the immune system of children with intestinal worms. We were pleased to observe, however, that the policy of treating all children in a school simultaneously for intestinal worms had worked really well. Nevertheless, our tests showed that half the children had intestinal parasites."

These pathogenic protozoa were Entamoeba histolytica, Entamoeba dispar and Giardia lamblia. The researchers found that many of the sick children who came to the research clinic seeking healthcare had these intestinal parasites. However, when the researchers examined children from a comparison population, they got a big surprise.

"The same percentage of children from the comparison population had these intestinal protozoa. However, since these children did not have any symptoms, this suggests that the protozoa they had do not make them sick."

Important to wait for the results

Unfortunately, one of these three protozoa is pathogenic. Entamoeba histolytica can cause amoebic dysentery. However, the researchers analysed stool samples by microscopy and could therefore not determine how many children had Entamoeba histolytica and how many had the harmless Entamoeba dispar.

"In the next project, we will examine the children using a polymerase chain reaction test to differentiate between Entamoeba histolytica and Entamoeba dispar. If we succeed, we can focus on treating the children with Entamoeba histolytica."

An obvious idea would be treating children collectively, similar to how the intestinal worms were combated. However, the treatment for protozoa is much more complicated because it has to be given several days in a row, whereas children with intestinal worms can be treated only once. In addition, according to Sebastian Leicht von Huth, there is another good reason to wait for the results instead of just starting to treat all children with protozoa.

"Protozoa can actually be beneficial in some cases. The harmless protozoa may even help to keep the children healthy. In any case, it is remarkable that so many children have them without getting sick. So the most important thing initially is to develop a method that can identify children with Entamoeba histolytica."

"Prevalence and potential risk factors for gastrointestinal parasitic infections in children in urban Bissau, Guinea-Bissau" has been published in Transactions of the Royal Society of Tropical Medicine & Hygiene. The study received funding from the Odense University Hospital Free Research Fund, Aase and Ejnar Danielsen Foundation, A.P. Møller Foundation of the Advancement of Medical Science and others. The Novo Nordisk Foundation has frequently supported the Bandim Health Project at Statens Serum Institut. In 2018, the Foundation awarded a grant to co-author Uffe Holmskov, Department of Molecular Medicine, University of Southern Denmark, Odense for the project FIBCD1-mediated Signal Transduction Pathways Regulate Gut Inflammation.

When genes make a difference

Content

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Your genes can predict whether you will gain or lose weight
Changing lifestyle has turned out to be a very difficult way to escape obesity. The human body has extremely strong mechanisms for retaining fat. This explains why researchers increasingly believe that the obesity epidemic will be conquered by learning to understand the mechanisms in our brain and fat tissue. Researchers mapped which genes are expressed in fat tissue – 13 years before they knew whether the participants gained or lost weight. The results show a clear profile and also indicate potential ways of helping the body lose weight.

Surgery can counteract genetically determined obesity
For decades, there has been debate on whether expensive gastric bypass surgery is worthwhile for people who are genetically predisposed to being severely obese. However, the evidence does not support the concern that these people eventually regain the weight after surgery. In fact, obese people who are less genetically predisposed to being overweight more often tend to remain obese after surgery. The researchers think that social and cultural factors could explain this.

Genetic testing can prevent suden cardiac arrest
When a person collapses with sudden cardiac arrest, genetic testing may determine whether family members are also at risk. Danish research shows how genetic testing and preventive treatment can save people's lives.

Researchers discover a link between genes and sudden cardiac arrest
Doctors know many lifestyle factors that increase the risk of sudden cardiac arrest. Nevertheless, many people at high risk live long without problems, whereas others die young. Researchers believe that genetic differences are the reason. Now a major research project with Danish participation has been the first to show a genetic link. The aim is to identify who are in acute danger based on their genes.

These genes influence whether you take risks

New research has discovered hundreds of genetic variants that are involved in determining whether you are prone to taking risks or playing it safe.

By Kristian Sjøgren

People's willingness to take risks varies enormously.

Some of us live on the edge or in the fast lane; others prefer the safe and familiar. The difference applies to both adventurousness and the general tendency to take risks but also to the propensity to engage in specific types of risky behaviour such as smoking, drinking alcohol, number of sexual partners and driving faster than the speed limit.

We are different.

A large genome-wide association study of more than 1 million people has identified hundreds of genetic variants that determine why you tend to take more risks than your neighbour or vice versa.

Not surprisingly, the research shows that these genetic variants especially affect one specific part of the body.

"We discovered that the genes affecting people's propensity to take risks are especially active in certain areas of the brain involved in making decisions and the brain's reward system by influencing the neurotransmitters glutamate and gamma-aminobutyric acid (GABA). The interesting thing about these transmitters is that they have opposing effects on the communication between our neurons. GABA inhibits the nerve signals and glutamate stimulates them. Our results suggest that the communication between the neurons plays an important role in people's tendency to take risks," explains the Danish participant in the study, PhD student Pascal Nordgren Timshel, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

"Our results suggest that the communication between the neurons plays an important role in people's tendency to take risks."

Pascal Nordgren Timshel

The new research results have been published in *Nature Genetics.*

Previously suspected biological pathways do not influence risk-taking

The researchers examined the factors that influence whether people are prone to taking risks.

The researchers thought initially that many biological systems and pathways would influence people's risk-taking . Specifically, the researchers focused on five main biological pathways: the steroid hormone cortisol, the neurotransmitters dopamine and serotonin and the sex hormones testosterone and estrogen.

Nevertheless, the researchers found no evidence of any association between risk-taking and the genes influencing the secretion of these neurotransmitters or hormones. They examined the genomes of the participants more broadly and found an association with the genes involved in neurotransmission with glutamate and GABA.

"Previous analysis was based on genetic information from a few hundred to one thousand people, and researchers specifically examined a few individual genes that were suspected of influencing risk-taking. Our study, in contrast, was not limited to specific genes but instead examined all genes in a study population of almost 1 million people," says Pascal Nordgren Timshel.

99 genetic variants associated with general propensity to take risks

In this bioinformatic study, the researchers manipulated very large data sets that link studies of the participants' whole genome with information about their self-reported propensity to take risks in general, self-reported adventurousness, smoking habits, alcohol consumption, number of sex partners and penchant for speeding.

The researchers could thus detect genetic variants that appear to increase risk-taking. These genetic variants arise when one or a few of the components of DNA are replaced by others. This slightly changes the function of the gene in which the DNA is located, and if the gene is involved in regulating the release of glutamate or GABA, then these genetic variants may affect risk-taking.

The researchers found several hundred genetic variants that increase people's tendency to take specific risks – including smoking or drinking alcohol – and 99 genetic variants that specifically increase people's general propensity to take risks.

The vast majority of these genes are associated with releasing GABA and glutamate in the brain.

"Our analysis indicates which areas of the brain specifically influence whether people are prone to take risks. We found, for example, that the genes that affect the propensity to take risks are especially active in specific areas of the prefrontal cortex, the area of the brain that regulates personality and decision-making. However, one problem in this study is that it had limited anatomical detail. The prefrontal cortex is a general designation, but we would like to be more specific," says Pascal Nordgren Timshel.

Understanding risk-taking and disease at the cellular level

Pascal Nordgren Timshel is working to improve understanding of how individual cells in the prefrontal cortex function to increase people's genetic tendency to take risks.

During his research project, he plans to use genetic analysis and bioinformatics to define the identity and function of cells more precisely.

This will improve the understanding of exactly what happens when people take risks and of how brain diseases and metabolic disorders develop.

"The long-term dream is to be able to apply my bioinformatic methods to a molecular atlas of all human cell types to understand the biology of disease at the smallest level: the cell. In fact, realizing this dream is not that far away," says Pascal Nordgren Timshel.

Pascal Nordgren Timshel is participating in the research project Human Cell Atlas, which aims to map all the thousands of types of cells in the human body.

"The human body comprises many different types of cells, and we know amazingly little about the diversity of these cells. Understanding the molecular mechanisms by which diseases develop requires mapping the identity and function of the cells involved. The Human Cell Atlas is the dawn of a new era in understanding cells that will become crucial for all aspects of biology and medicine."

"Genome-wide association analyses of risk tolerance and risky behaviors in over 1 million individuals identify hundreds of loci and shared genetic influences" has been published in Nature Genetics. Several of the authors are employed at the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

How the genes of the mother and child influence birthweight

Babies who are especially small or large at birth have not only a higher risk of complications at birth but also a greater risk of overweight, type 2 diabetes and high blood pressure in adulthood. Using a new revolutionary genetic method, researchers have succeeded in understanding how much the genes of a child and mother and the environment in the womb determine a child's birthweight. According to the researchers, the new method can potentially help the parents of children at risk in giving their children a better start to a long and happy life.

By Morten Busch

Children inherit half their genes from their mother and half from their father. This genetic mix creates the unique characteristics of a child – both positive and negative. Some children have genes that limit their early growth or increase it more than normal. These metabolic effects can last their whole life. Helping children early in life can potentially give them a healthier and longer life. However, understanding the relationship between birthweight and health risks requires understanding and being able to distinguish between the genetic and environmental causes.

"The new method can separate these effects for the first time. Three quarters of the genetic effects on birthweight originate from the child's genes; maternal genes, which also affect the environment in the womb, account for one quarter. This new method will potentially enable us to screen the children and parents so that we can help prevent lifestyle-related diseases at an early stage in life," explains Jens-Christian Holm, Clinical Research Associate Professor, Department of Clinical Medicine, University of Copenhagen and consultant, Department of Paediatrics, University Hospital Holbæk.

New pieces of the puzzle

The researchers in this large international research project compared the genetic information of more than 300,000 mothers and the birthweight of one of their children. Using new statistical methods, the researchers successfully separated the effects of the genes of the mother and the fetus on the birthweight of the newborn infants. The research involved more than 200 international clinics from 20 countries participating in the Early Growth Genetics Consortium or the UK Biobank study.

"We conclude that the direct effects of a baby's genes are the most important factor influencing birthweight. However, the mother's genes that were not passed on to the baby provide about one quarter of the genetic effects. These genes affect the growth of the fetus by influencing the environment in the womb during pregnancy, including the amount of glucose supplied, which directly determines how much the fetus grows."

In addition to determining the ratio between the effects caused by the genes of the fetus and the mother, the research also identified 190 independent association signals, of which 129 are new. This means that the researchers have obtained numerous new pieces of the puzzle that can help explain the relationships between genes and birthweight.

"These 190 association signals can potentially also be used to screen children for their risk of metabolic disorders, and if we can also understand the specific effects of the genes, we can help the children so that they can, for example, eat in a certain way and avoid becoming overweight. Further, understanding the mother's genes may enable us to stabilize her glucose metabolism, which strongly affects the environment in the womb. The perspectives are really striking."

Interestingly, the researchers also found numerous association signals involving both the genes of the mother and child. They sometimes reinforced each other and sometimes opposed each other.

"For example, some genetic effects raise the mother's blood glucose, making the baby bigger because the fetus produces more insulin in response. However, if the child inherits the same genetic variants, this reduces the amount of insulin the child produces, limiting growth and counteracting some of the mother's growth-promoting effects."

A completely new way of thinking

The study is the largest of its kind so far and results in new insight into the complexities of how the genes of mothers and babies interact and affect birthweight. In addition, the study adds a new chapter to a previous study published in Nature in 2016, in which the researchers helped to identify 60 genes that influence birthweight, type 2 diabetes and cardiovascular diseases.

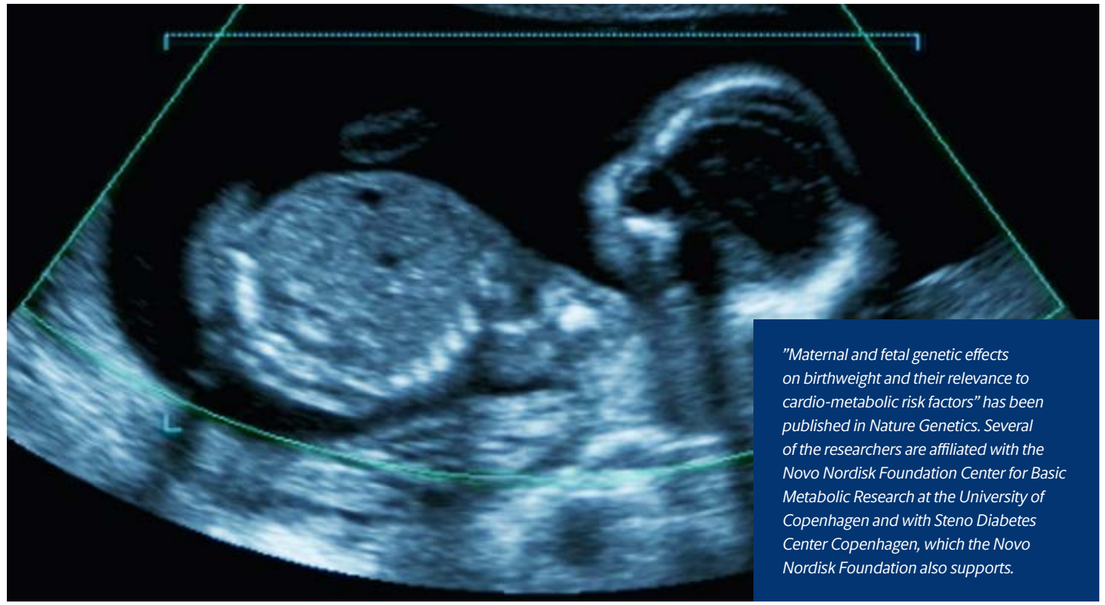
"In December 2018, we published a study from University Hospital Holbæk involving 920 overweight children whom we screened for combinations of 15 relatively frequent genes linked to overweight among children to determine whether our treatment is effective. The good news was that, no matter which or how many of these 15 common genes they had, we could help them lose weight and reduce their previous signs of metabolic disorders."

Evidence indicates that a child's genetics primes them to absorb too much or too little energy. By understanding the factors that affect birthweight among babies born very large or very small, Jens-Christian Holm hopes that we will be able to reduce children's risk of obesity and its complications later in life.

"This is a completely new way of thinking, and the potential presents new perspectives. A new type of precision medicine that enables us to advise parents and children early. This could mean that some children need to be especially physically active or that the parents must pay special attention, for example, to sugar and fat given the genetic challenges the children face."

Jens-Christian Holm understands the ethical implications of screening children based on their genes, but not screening them also has ethical implications if the children can otherwise be helped to live a longer and healthier life.

"There are naturally pitfalls in this, so we must thoroughly discuss both the technical issues of false positives and false negatives if we can identify a high-risk group but also the ethical aspects. However, I think this cuts both ways, and we owe future generations to seriously discuss the opportunities."



"Maternal and fetal genetic effects on birthweight and their relevance to cardio-metabolic risk factors" has been published in Nature Genetics. Several of the researchers are affiliated with the Novo Nordisk Foundation Center for Basic Metabolic Research at the University of Copenhagen and with Steno Diabetes Center Copenhagen, which the Novo Nordisk Foundation also supports.

Genetic test can detect men at greatest risk of developing prostate cancer

Prostate cancer is the most heritable type of cancer. However, little is known about which genes cause some men to have a high risk of developing prostate cancer. A total of 130 research groups worldwide have now joined forces to analyse the genetic data of thousands of people with prostate cancer. The results suggest that one region of the genome is as strongly associated with prostate cancer among men as another region is associated with breast cancer among women. This discovery paves the way for targeted screening of men with an unfavourable genetic profile, similar to screening for breast cancer.

By Morten Busch

Prostate cancer is the most common type of cancer affecting men, with more than 160,000 men diagnosed annually worldwide. In addition, prostate cancer is also one of the most heritable types. However, men are not screened for the disease simply because no screening methods are reliable enough. Researchers have collected new genetic data from more than 70,000 men with prostate cancer and compared the data with those from more than 52,000 healthy men.

"Previous studies identified more than 100 gene variants that each slightly increases men's risk of developing prostate cancer – but combined, they can have a considerable effect. The previous studies especially identified gene variants in a region of the human genome called chromosome 8q24. However, its significance has not been explored in detail. By analysing even more people with prostate cancer than before, and in greater detail, we have now succeeded in zooming more closely into this region," explains Karina Dalsgaard Sørensen, Professor with Special Responsibilities, Department of Clinical Medicine, Aarhus University.

Fourfold greater risk

The researchers have thus identified 12 very specific gene variants that may explain 25% of the heritability of prostate cancer. Combined with the existing knowledge in the field, 40% of the heritability of prostate cancer can now be explained.

"We can therefore also develop a genetic test that can reliably identify the men who have a high risk of developing prostate cancer and then ensure that they are offered regular check-ups."

The study was a genome-wide association study that compared genetic data from many men with and without prostate cancer. The genetic material was comprehensive and of high quality because 130 research groups globally pooled data from their individual studies.

"We could therefore identify the most frequent gene variants among men with prostate cancer. We also zoomed in on the genetic variation in the chromosome 8q24 region, which previous studies had shown contains many of the gene variants."

Gene variants on chromosome 8q24 are associated with several types of cancer, including prostate cancer. The researchers found 12 genetic risk variants for prostate cancer within a relatively small region of the genome.

"The results show that men with many of these variants have a fourfold greater risk of developing prostate cancer. Cumulatively, these 12 variants comprise 25% of the total genetic risk."

Will also investigate circulating tumour DNA

In addition to the genetic factors, age and lifestyle are also important in determining who develops prostate cancer, similar to other types of cancer. The reason this study is especially relevant is that the genetically determined risk in prostate cancer is estimated to be 40%, which is much greater than for other types of cancer.

"Now that we have identified 12 important variants, the next step is trying to understand their biological function. Why do the variants in this region trigger prostate cancer? Understanding this may enable us to find pharmaceutical targets for new anti-cancer treatments or methods to prevent the cancer from developing."

The studies also enable other types of studies on which Karina Dalsgaard Sørensen and her Danish colleagues are focusing. They will try to understand how prostate cancer metastasizes (spreads to other sites in the body) and why it can resist treatment. The genomic data are especially important for this.

"Prostate cancer only becomes dangerous once the cancer metastasizes and resists treatment. We are thus focusing on discovering and sequencing the circulating tumour DNA in the bloodstream because it has leaked from the tumour cells. If we can achieve this, we may also be able to understand the underlying mechanisms that develop resistance and perhaps develop drugs and treatment strategies that can inhibit these mechanisms."

Men follow recommendations

In addition to potentially determining the mechanisms enabling prostate cancer to develop in the long term, this new knowledge may help now in detecting cancer early so it can be treated and cured before it metastasizes. Men are not currently routinely screened for prostate cancer because the screening methods are simply not reliable enough. An existing screening method measures the prostate-specific antigen (PSA) concentration in the blood.

"Because PSA is present naturally in men, a man with a naturally high level of PSA may be diagnosed with prostate cancer and therefore may be treated unnecessarily. However, if we assess a man's risk based on his genetic profile, we can confine testing to the men with the greatest risk of developing prostate cancer. They can then be routinely checked by measuring their PSA concentration."

The Danish researchers are testing this method in a research project in the Central Denmark Region in which 5000 men have volunteered to undergo genetic testing. The men with the least favourable genetic risk profile (threefold greater risk) will then be offered regular PSA tests. The first 4000 men have already been tested, and the results are very promising.

"Although the study is still ongoing, we have already had initial success: the men in the high-risk group are actually following the recommendations made after the genetic testing. This is a very positive initial result because experience shows that men consult a doctor less often and later."

Another study of prostate cancer diagnosis is underway in the Central Denmark Region. In this trial, selected doctors are evaluating the Stockholm-3 (STHLM3) test, a new multistep blood-based test used in Sweden that can detect the early development of prostate cancer more precisely than the standard PSA test. The trial began in 2018 and is expected to run for 2 years.

"The new blood test considers more and different parameters than the PSA result, including age and heritability through genetic testing. In this respect, the new blood test is a far more personalized method of detecting prostate cancer in the future, and we are very optimistic and excited about the results of this research project."

"Germline variation at 8q24 and prostate cancer risk in men of European ancestry" has been published in Nature Communications. In 2016, the Novo Nordisk Foundation awarded a grant to a co-author, Karina Dalsgaard Sørensen, Professor with Special Responsibilities, Department of Clinical Medicine, Aarhus University, for the project Genome-Wide CRISPR-Cas9 Screening for Drug Resistance Mechanisms and Identification of Novel Predictive and Monitoring Biomarkers for Castration Resistant Prostate Cancer.

Certain genes ensure that exercise benefits health

The health benefits of exercise are hardly breaking news. However, we still know very little about whether physical activity benefits everyone's health equally. Previous research has suggested more than 100 genes in the human genome that could link exercise to our health. An international research team has now significantly narrowed the field down and especially focuses on four genes. The researchers hope to be able to help people who have difficulty in exercising enough to improve their health.

By Morten Busch

Many people know the feeling of looking outside on a wet grey day and considering whether they would really benefit from going for a run or cycling to the gym. Actually, this is an excellent question, because although exercise has many benefits, they vary depending on our genes. A study of 250,000 people from five continents shows that genes are crucial for determining how physical activity affects the quantities of fatty substances (lipids) in our blood.

"We knew that genes are associated with the quantities of lipids in the blood, but we were not certain how exercise modifies the genetic effects. We found four gene loci at which exercise strongly interacts with the regulation of the quantities of lipids in the blood. We will now try to understand the mechanism so that we can possibly mimic the effect and thus benefit people who have difficulty in exercising enough to improve their lipid levels," explains a main author, Tuomas Oskari Kilpeläinen, Associate Professor, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

Cholesterol rising and falling

The new study gathered data from 86 original studies conducted on five continents. Not surprisingly, the interaction between genes, physical activity and blood lipids is extremely complex. Each original study alone was too small to find any genes that are important for this interaction, but the large international research team could get a much clearer picture by pooling the existing studies.

"Systematizing the studies so that we could, for example, compare physical activity between the studies was a major operation. Further, there are millions of genetic variants, so it was hard to find which genes are important by looking at each of these studies individually. However, when we aligned the studies, we found that responsiveness to exercise was consistently linked to four genetic loci."

The lipids the researchers examined were the two types of cholesterol – high-density lipoprotein (HDL) and low-density lipoprotein (LDL) – and triglycerides. All three lipids are significant risk factors for cardiovascular diseases, and the body therefore needs to maintain these lipids in appropriate quantities.

"We found four gene loci at which exercise interacted with the regulation of blood cholesterol. Increased physical activity interacts with the three lipid-regulating genes CLASP1, LHX1 and SNTA1, and this results in a higher level of HDL, sometimes called "good" cholesterol. Exercise also regulates another gene, CNTNAP2, to make the body produce less of the harmful LDL cholesterol."

Want to mimic the mechanisms

The new results may prove to be important for several reasons. First, after narrowing down the gene candidates considerably, researchers can now start studying why these genes seem to respond strongly to exercise.

"This will potentially enable us to find the people who can derive the most benefit from exercising, because their genes simply respond best to exercise and thus optimally affect their blood lipids. Conversely, other people may need higher levels of exercise or other methods to achieve the same benefits."

Whether such knowledge can be achieved depends on future studies. Now that the researchers have established the interaction, much work lies ahead for understanding the mechanism behind it. The first step involves working with the genes in cell cultures in the laboratory to confirm the interaction and to explore how the expression of the four genes changes.

"In addition to understanding what is happening at the molecular level, we also want to examine what happens over time to individual people. Does the effect of the gene variants change over time, with exercise providing more benefits, for example, when a previously sedentary person engages in exercise training? The ultimate goal is naturally to understand the mechanisms so well that we can, for example, mimic them pharmaceutically, thereby helping people who cannot get enough exercise to improve their lipid levels."

"Multi-ancestry study of blood lipid levels identifies four loci interacting with physical activity" has been published in Nature Communications. In 2010, the Novo Nordisk Foundation awarded a grant of DKK 885 million to the University of Copenhagen to establish the Novo Nordisk Foundation Center for Basic Metabolic Research, where Tuomas Oskari Kilpeläinen is employed as an Associate Professor.

Ancient proteins are the new hot property in science

The past does not simply hold details about our history. Knowledge on human evolution can also provide important information that can be used to combat the development of disease now. The use of prehistoric DNA has revolutionized evolutionary research and archaeology in the past 30 years. Unfortunately, ancient DNA is not always well preserved. A new study of relationships between sloths has been published in one of the world's most prestigious journals – not because of the sloths but more because its analysis of prehistoric proteins will revolutionize science.

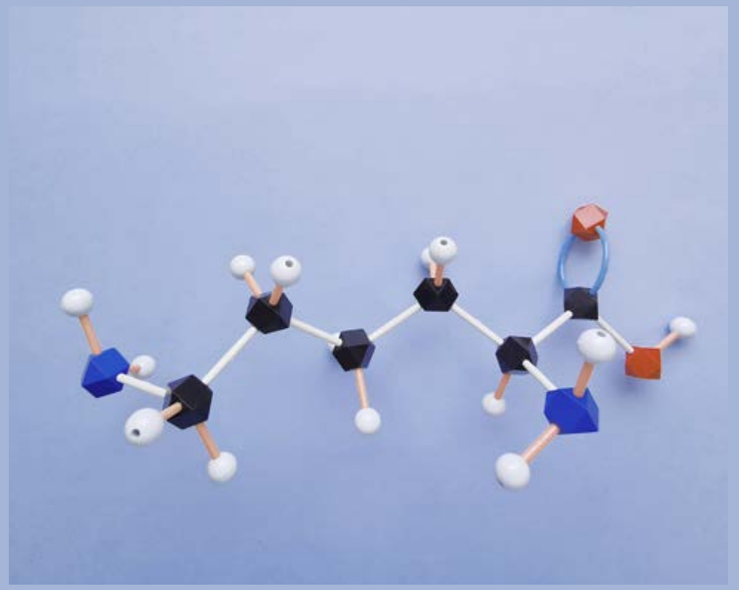
By Morten Busch

A new technology enables researchers to step further back in time by a factor of up to 10-fold because proteins remain intact that much longer than DNA. Excavations of prehistoric sites are therefore much more likely to find intact protein than DNA. The great technical advances in mass spectrometry enable researchers to decode sequences from proteins that are millions of years old, and this has already solved prehistoric mysteries.

"Our latest study of the early evolution of sloths shows the potential of the new technology. Biologists can spend decades assessing kinship based on bone size, structure and shape, but we can determine the kinship in hours or days based on a very few protein samples. We even discovered that the morphological conclusions were wrong. So this technology enables us to answer questions that DNA has been unable to solve," says a co-author, Matthew Collins, Danish National Research Foundation Niels Bohr Professor, Evogenomics Group, Globe Institute, University of Copenhagen and McDonald Chair, Institute of Archaeological Research, University of Cambridge, United Kingdom.

Powerful method
Today, only two types of the Folivora suborder of sloths still exist, but back in the Cenozoic Era 1.8 million years ago, sloths were widespread in many parts of the Western Hemisphere. However, because many of these animals lived in humid and hot areas, which have an unfavourable climate for preserving DNA, researchers have not been able to identify the relationships between the types of sloths using current methods.

"We managed to obtain 120 samples from 24 species. Of these, we found enough protein for analysis from about one third. We then used mass spectrometry to determine the sequence of the same protein, collagen, in each sample. Then we could start the process of examining the kinship relationships of the sloths based on the sequences."



"This technology enables us to answer questions that DNA has been unable to solve."

Matthew Collins

The researchers were then surprised to discover that the two-toed sloth of the Choloepus genus and the three-toed sloth of the Bradypus genus originated from two different families of the Folivora sloth suborder: Choloepus from the Mylodontidae family and Bradypus from the Megatheriidae family.

"Previous morphological studies suggested that Choloepus and Bradypus were more closely related. This new method provides a more accurate picture than is possible from morphological studies alone. The protein sequences are not as rich as DNA, and are more prone to homology. In a way, it is like unearthing extra skeletal elements with new bits of evidence."

Evolution mitigates catastrophic effects
Although the new method has many possible new applications, it works best together with DNA analysis. Protein analysis can detect intact proteins in more places and further back in time, but DNA analysis provides more information.

"DNA analysis provides both sequences that encode proteins and sequences that are never translated into proteins. The DNA code may also change without this changing the protein encoded by the DNA. This is because several three-letter codon combinations in the DNA code get translated into the same amino acid in the final protein."

For example, the codons GGT, GGC, GGA and GGG are all translated into the amino acid glycine. So, although the DNA code may change, the protein may not.

"Evolution also tends to mitigate the effects of some of these mutations in the DNA that arise through natural evolutionary changes, often because mutations in the equivalent proteins would have catastrophic effects on the organism."

Enormous potential
The proteins thus change much less than DNA, but prehistoric proteins also contain much less information than prehistoric DNA.

"Nevertheless, combining protein and DNA techniques works very well. DNA technology is very accurate, but we can find protein in many more locations, which provides greater depth in the analysis."

According to Matthew Collins, protein analysis has far greater development potential for new lines of investigation in archaeology and heritage science than the more mature field of DNA sequencing because most objects made of biological material have proteins.

"We are only now realizing how great the potential is as we discover how far back in time proteins have survived, where we find them and especially how much information we can extract from them. In addition, we can investigate more than our prehistory. This knowledge can also be used in the present era, such as in investigating ageing."

"Palaeoproteomics resolves sloth relationships" has been published in Nature Ecology & Evolution. Co-author Jesper Velgaard Olsen is Deputy Center Director and Professor, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

Epigenetics and childrens birthweight

Birthweight is associated with health outcomes throughout life. For example, children who are born heavier or lighter than average have a higher risk of developing diabetes and cardiovascular diseases. A new large study reveals that epigenetics is closely associated with children's birthweight.

By Kristian Sjøgren

"Meta-analysis of epigenome-wide association studies in neonates reveals widespread differential DNA methylation associated with birthweight" has been published in Nature Communications. Researchers from the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen contributed to the study.

A major international research project has now shown that birthweight is closely associated with epigenetic alterations in the child's DNA. As the fetus develops, specific epigenetic molecules switch genes on and off, making them accessible or inaccessible to the molecular machinery of cells. This influences the weight of newborn babies.

This new large meta-analysis included almost 1000 children in Denmark from the Danish National Birth Cohort (Better Health in Generations).

"The study has been designed to be large to enable the researchers to see the statistical links that are not visible in smaller studies because epigenetics varies randomly between humans. Carrying out such a large study can provide greater insight into the molecular biological mechanisms that link birthweight with possible influences on the fetus through the mother, such as smoking, which changes birthweight," explains a Danish contributor to the international research project, Thorkild I.A. Sørensen, Professor, Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen.

The study was published recently in *Nature Communications*.

Small molecules alter DNA

The international study included 8825 newborns from 24 cohorts in the Pregnancy and Childhood Epigenetics Consortium. The researchers studied the DNA in blood samples taken from the umbilical cords of newborns to map where the methyl groups are located in the newborn child's DNA.

Methyl groups are small molecules that attach themselves to DNA at sites at which cytosine-guanine base pairs are present. The genome has millions of these, and the methyl groups bind to cytosine, thereby changing the opportunities the cells' molecular machinery has to decode the DNA. This often means that genes are silenced.

As the fetus develops, epigenetic alterations constantly switch various genes on and off to very precisely orchestrate the formation of the fetus. The result, if all goes well, is that the fetus grows and develops the various organs, tissues and brain in a very predictable pattern that ultimately results in the birth of a healthy child.

Epigenetic differences and variation in birthweight

The new study shows that DNA methylations are associated with differences in children's birthweight at 914 sites in the genome.

The more methylations the fetus has at any of these 914 sites on the genome, the greater the average birthweight. Conversely, average birthweight is lower if there are more methylations elsewhere in the genome.

The researchers deduced that a 10% decrease or increase in the degree of methylation is associated with a birthweight difference of between 183 grams lower and 178 grams higher than average.

"The DNA activity during fetal development influences a child's birthweight, and epigenetics plays a role. We can therefore link the activity at the 914 sites in the genome with their birthweight," says Thorkild I.A. Sørensen.

Understanding fetal development better

Thorkild I.A. Sørensen explains that the study goes deeper towards understanding what happens at the DNA level as the fetus develops.

Once the researchers have obtained an overview of how activating or deactivating various genes influences birthweight, they hope that this will enable them to understand the risk of developing numerous chronic diseases and perhaps reduce the risk of later disease at this early stage in life.

"In the long term, we can improve our understanding of how fetal development affects children's health for the rest of their lives. One example might be that, in the future, we hope to see which genes are switched on and off as the fetus develops for a child who later develops asthma, obesity, diabetes or another disease. In addition to the genetic differences between people, this is the overall key to the differences between us," says Thorkild I. A. Sørensen.

Smoking alters the methylation of DNA

The big question is: what affects the epigenetics of a fetus?

The more researchers understand how epigenetics affects children's risk of being born overweight and their risk of developing various diseases, the better they can also understand

"In the long term, we can improve our understanding of how fetal development affects children's health for the rest of their lives."

Thorkild I.A. Sørensen

how environmental factors influence epigenetic alterations and cause problems.

Smoking is one example.

Everyone knows that smoking is unhealthy for mothers but especially for their unborn children, who end up with a lower birthweight. Pregnant women should therefore definitely not smoke.

Research has already shown that a mother's smoking during pregnancy changes the methylation of the child's DNA, and this is probably why children whose mothers smoke are at increased risk of being born underweight.

"Many environmental factors influence the epigenetics of the fetus, and smoking is just one. Obesity is another, but exercise, alcohol consumption, eating habits and various chemicals can also affect the activity of various genes as the fetus develops. We have much more to discover," says Thorkild I.A. Sørensen.

This new study is a meta-analysis of data from 24 birth cohorts. One was the Danish National Birth Cohort, which included 100,000 pregnant women, with blood samples being taken from the umbilical cords of the newborns of about 60% of these women. The international study included almost 1000 children from the Danish National Birth Cohort.

In addition to Denmark, Norway was a major contributor to the study, with three cohorts totalling almost 2000 children.

The path to better treatment

Content

See also the following articles at **sciencenews.dk**

Asthma prevention begins before birth
Asthma is the most common chronic disease in childhood and the most common reason children are hospitalized. The 2019 Novo Nordisk Prize recipient, Hans Bisgaard, found that giving fish oil to women during pregnancy reduces the risk of their children getting asthma by 30% and significantly more if they have a certain genetic profile. His latest result shows that fish oil also influences the development of the child's nervous system, including cognition.

Danish researchers develop many candidates for promising antidiabetes target
For many years, researchers have known that a specific receptor is an ideal target for developing candidates for medicine to combat diabetes, but so far all efforts have failed. Now Danish researcher Thomas M. Frimurer and his group have used computer simulation to develop a series of new potential medicines to treat diabetes.

How researchers plan to design better medicine
Medicine often has difficulty in penetrating the barrier between the gut and the bloodstream, and many types of medicine therefore need to be injected subcutaneously or intravenously. Researchers from the Technical University of Denmark are working on developing new methods to enable the body to absorb medicine as pills and to develop drugs that boost the immune system.

Migraine medicine: We only buy if it is effective
More than one in seven people have migraine in Denmark, costing billions of kroner in treatment and lost earnings. Nevertheless, little is known about which types of migraine medicine are effective for each individual. By linking data from the Danish National Prescription Registry with information about symptoms among people with migraines, researchers have now clarified that people – perhaps naturally enough – only buy the medicine if it is effective. This new knowledge can identify the people for whom the medicine is not effective to enable them to find alternative treatment.

After 60 years:
”Now we finally understand how this medicine works”

For 60 years, metformin has been doctors’ first-line drug for treating people with type 2 diabetes. However, no one has known precisely how it works. An international research team with Danish participation has now discovered how metformin works. This pioneering discovery will help not only the 400 million people with diabetes but also potentially people with other conditions.

By Morten Busch

When people swallow a pill, the medicine inside has been thoroughly tested to ensure that it works as intended and has no serious side effects. Nevertheless, scientists do not always know how medicine works. This can be a problem for people taking several types of medicine, because predicting the effects is more difficult. A new study has finally discovered the how the diabetes drug metformin works.

“Metformin behaves differently to many other drugs. One reason is that only a few organs absorb it, and it is not metabolized in the body. People simply excrete it in their urine once it has worked. Precisely tracking where and how it works has therefore been difficult. Our new research shows that metformin works by targeting energy-sensitive proteins in the liver, and this interaction reduces the liver’s production of glucose,” explains co-author Niels Jessen, Professor of Clinical Pharmacology, Aarhus University and Head of Research, Steno Diabetes Center Aarhus.

Clear effect

Previous research has shown that metformin works by suppressing the production of glucose by the liver, thereby reducing the symptoms of type 2 diabetes. However, because metformin passes unaltered through the body, determining precisely how this happens has been difficult. One of the many theories was that metformin affects the liver’s energy balance and thereby slows down the production and release of glucose.

“To test this theory, we tried to see what would happen if we removed one of the key components of the liver’s energy metabolism: an enzyme called FBP1. We therefore slightly modified the enzyme so that it was no longer sensitive to adenosine monophosphate (AMP) that usually suppresses FBP1, causing it to produce and release less glucose.”

This also enabled the researchers to examine whether FBP1 and AMP are actually essential for the effects of metformin. If the theory was correct, metformin would continue to alter the energy balance so that more AMP would be produced and, since the mutated FBP1 enzyme would be insensitive to AMP, metformin would no longer be able to reduce the production and release of glucose.

“We demonstrated this effect in the laboratory, so it was very exciting to see what would happen when we treated mice that had the altered enzyme with metformin. The effect was clear, substantially impairing the ability of metformin to reduce high blood glucose.”

Unknown effects create anxiety

Based on the experiments, the researchers concluded that metformin works by creating mild energy stress in the liver, which increases the quantity of AMP, which then suppresses FBP1 and thereby the production and release of glucose. Even though the researchers definitely cannot rule out other effects of metformin, this new study is a milestone in understanding this type of diabetes treatment.

“Although we still do not understand precisely how metformin creates energy stress, the discovery of the specific influence on one of the liver’s proteins is pioneering and will be very important for using metformin to treat people in the future.”

The new knowledge will help to solve one of the specific challenges associated with modern medicine: people often take several different types of medicine simultaneously. If doctors do not understand how an individual drug works, predicting how the drugs will interact will also be very difficult.

“Unknown effects make doctors anxious. We can now predict these effects better, and this will clearly improve treatment because we can now ensure that we can dose and combine different types of medicine correctly.”

This knowledge will also assist in developing precision medicine (sometimes called personalized medicine), in which the medicine is tailored based on the genetic profile of an individual. Knowing which genetic version of FBP1 a person has will enable doctors to predict whether metformin will work and what dose to prescribe.

“This is a good example of the importance of funding basic research. Metformin is a 60-year-old drug with a long-expired patent, so no company is interested in researching how the drug works. This type of research is only enabled if public research is funded through independent research grants. And this research has now resulted in being able to develop more targeted and effective medicine with fewer side-effects.”

“Metformin reduces liver glucose production by inhibition of fructose-1-6-bisphosphatase” has been published in Nature Medicine. Niels Jessen is Head of Research of Steno Diabetes Center Aarhus, which has been partly funded through a donation from the Novo Nordisk Foundation.

Gastric surgery works: Let's turn it into a pill

The obesity epidemic rages. Every sixth person is extremely obese today. One of the few effective ways of fighting obesity is surgery. Previously it was thought that the people lose weight after surgery because the stomach has less room for food. However, new research shows that people lose weight because of major changes in hormone balance. A new study has charted how the surgery affects the body. The goal is now to recreate this effect by using a pill to avoid surgery.

By Morten Busch

Gastric bypass surgery is a simple procedure that channels food directly from the upper part of the stomach to the lower part of the intestine, thus eliminating most of the function of the stomach and intestines. This helps most people lose considerable weight and avoid life-threatening diseases such as type 2 diabetes. The reason they lose this weight, however, differs completely from what was expected. Danish researchers have charted the physiological changes resulting from gastric bypass and the more recent gastric sleeve surgery.

"The surgery drastically changes the secretion of hormones. Bypass surgery increases the secretion of the appetite-inhibiting hormones, whereas sleeve surgery reduces the secretion of appetite-stimulating hormones. Our ultimate goal is to mimic these changes without having to do the surgery," explains co-author Maria Saur Svane, postdoctoral fellow, Hvidovre Hospital, Denmark.

Reducing appetite

Gastric bypass surgery changes the gastrointestinal anatomy so that food flows directly to the remote part of the small intestine and bypasses most of the stomach. A sleeve gastrectomy removes most of the stomach, leaving a narrow passage for food through a small tube.

"These two procedures differ considerably in the rate at which nutrients are absorbed from the food eaten and in the secretion of gut hormones."

The researchers determined the absorption of nutrients and secretion of gut hormones by combining intravenous stable isotopes of glucose and amino acids combined with giving patients a meal also containing stable isotopes. After the patients ate the food, the researchers then followed the rate at which the isotopes were absorbed and the quantity of hormones secreted by the gut.

"Bypass surgery increases the secretion of the appetite-inhibiting hormones from the remote part of the gut, such as GLP-1 and PYY, whereas sleeve surgery does not substantially increase the secretion of these hormones. Instead, sleeve surgery reduces the secretion of hormones from the gut, such as the appetite-stimulating hormone ghrelin, which is completely suppressed."

Eliminating diabetes

The most important reason for gastric surgery is major weight loss, but an additional advantage is eliminating diabetes. People undergoing surgery do not eat much during the first couple of days afterwards, which means they start losing fat in the liver. The fat disappears from the liver after only a couple of days.

"This turns out to be vital for the liver regaining its sensitivity to insulin. Insulin can again reduce the glucose production in the liver, and that is one of the essential mechanisms through which diabetes can occur," explains co-author Jens Juul Holst, Professor, Novo Nordisk Foundation for Basic Metabolic Research, University of Copenhagen.

The second important thing that happens is that, once people start to eat again, the gut is stimulated differently than before surgery. The food enters further down in the small intestine, stimulating many new endocrine cells. This increases the release of hormones that strongly affect insulin secretion at the same time that the amount of glucose from the food increases substantially.

"Together, these two things mean that people produce a lot of insulin. So now they have regained insulin sensitivity and have increased insulin secretion, and this is essential for these people with diabetes. That was their problem. They were insensitive to insulin and they had too little of it, and all of a sudden both these things are repaired after surgery, and this only takes a few days."

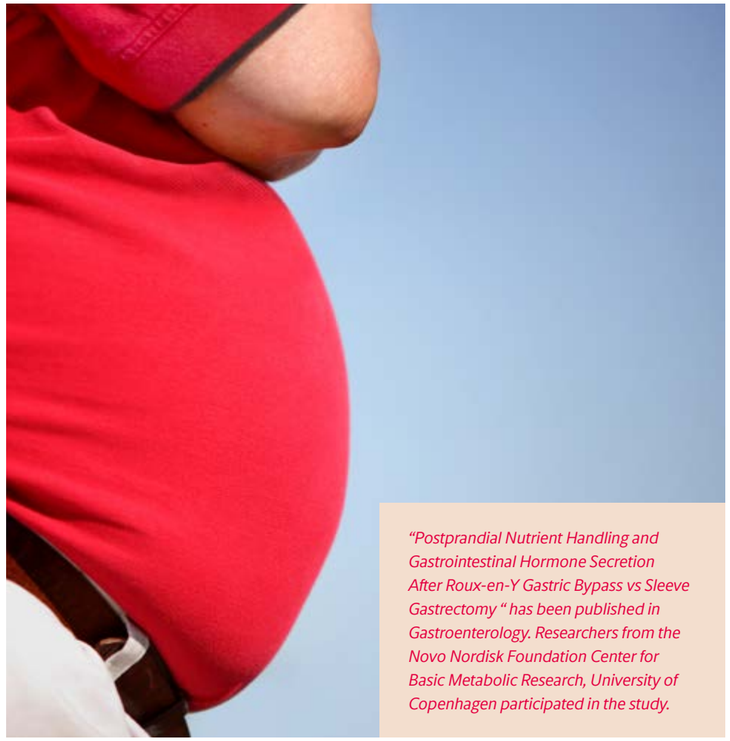
Using nature's own mechanisms

Although the two surgical procedures, gastric bypass and sleeve gastrectomy, are anatomically very different, their clinical performance in weight loss and diabetes remission seems to be similar. However, the new study shows that the physiological effect of the two procedures differs completely in terms of the rate at which nutrients are absorbed and the rate at which the gut secretes hormones.

"These types of surgery are expensive, they are risky and the patient needs lifelong vitamin replacement and follow-up by doctors. The optimal goal for us is to fully understand the mechanisms behind these favourable effects to mimic these changes without performing the surgery."

Both gastric bypass and sleeve gastrectomy cause most people to lose considerable weight and eliminate their type 2 diabetes. GLP-1 is one of the hormones whose secretion is especially increased after such surgery. This has already been formulated into a drug. In the future, the researchers hope to combine the different effects of different hormones to provide even more favourable clinical benefits.

"Our ultimate goal is to use these hormonal changes surgery causes to create a new medicine. This means using nature's own mechanism for reducing food intake and inducing weight loss. Since more than one hormone is involved in causing weight loss after surgery, we hope to combine them and achieve even more synergy."



"Postprandial Nutrient Handling and Gastrointestinal Hormone Secretion After Roux-en-Y Gastric Bypass vs Sleeve Gastrectomy " has been published in Gastroenterology. Researchers from the Novo Nordisk Foundation Center for Basic Metabolic Research, University of Copenhagen participated in the study.

How life's building blocks got their freedom of movement

The myriad processes that take place in our body's cells are the basis of life as we know it. Since cells are filled with fluid, British chemist Carol Robinson created a lot of attention by setting out to investigate life's processes in a vacuum. Despite massive resistance, she persevered. Now she is receiving the Novozymes Prize for founding a new subfield of mass spectrometry to investigate the shape of proteins and how they interact. Today, this technique is used to identify brand-new targets for drugs.

By Morten Busch

Some of the most important proteins in our bodies are on the surface of all cells. The membrane proteins control the transport in and out of cells and are key to how cells communicate. This makes membrane proteins hugely important drug targets. However, since the proteins are situated inside hydrophobic membranes, researchers have had great difficulty in figuring out how the proteins assemble and interact with other molecules.

Carol Robinson is receiving the 2019 Novozymes Prize for her scientific breakthroughs in using mass spectrometry – especially her pioneering work on using mass spectrometry for analysing protein complexes.

"The membrane proteins are the really critical complexes and are incredibly hard to study, because of this whole phase issue, where they sit in the oily phase. What we did was to coat them in detergent – effectively in soap bubbles – and then we release them into a mass spectrometer. Miraculously, they stay intact in a folded stage, so we can examine their 3D structure but also how they bind to other proteins or lipids," explains Carol Robinson, Professor, Department of Chemistry, University of Oxford.

An unusual path to success

Mass spectrometry was originally developed to determine the mass of small molecules. In a mass spectrometer, the molecules are ionized and deflected in a magnetic field during their flight. Heavy molecules are hardly affected in their flight, whereas lighter ones are deflected more. Measuring how far the ions fly can determine how much they weigh. The molecules are sorted based on their mass-to-charge ratio.

"I am still hugely fascinated with seeing a beam of ions fly through a mass spectrometer and what you can learn from the spectra. A mass spectrometry spectrum is a bit like a sudoku puzzle. You start to get all possibilities and then you can solve the problem and you feel tremendously satisfied."

Carol Robinson ending up as the first woman chemistry professor at Oxford was not a given, and her journey has been quite unusual. After leaving school at 16, she joined Pfizer as a technician working in the mass spectrometry laboratory. One day, one of Carol's colleagues recognized that she had very special potential.

"They encouraged me to do seven years of part-time study. It was the long haul, and then I was delighted to be accepted at the University of Cambridge to do a PhD. Traditionally, mass spectra were just used to determine mass. In my PhD project, I was looking at how we could also use the mass spectrometer to determine the sequence of small fragments of a protein."

Molecular elephants with wings

Since proteins are built from specific sequences of the 21 amino acids – 21 different building blocks with different masses – the researchers managed to identify the protein sequences by looking at the distance between the peaks in the spectra. Carol Robinson's career was on track, but then something happened.

"I am still hugely fascinated with seeing a beam of ions fly through a mass spectrometer and what you can learn from the spectra."

Carol Robinson

"I did something that was considered rather unusual. I took a career break for eight years, which was bit unconventional in those days. I really enjoyed the time at home with my three children and then went back to Oxford."

At 35 years old, she returned to science through a junior position working on mass spectrometry in Chris Dobson's group at the University of Oxford. While she was away, some revolutionary things had happened in mass spectrometry.

"I was encouraged to enter the new field of proteomics that emerged at that time. But we were not just looking at peptides and sequencing the amino acids along small chains. We were now looking at whole proteins, which are huge in terms of mass spectrometry."

Instead of being just a few hundred mass units (daltons), the proteins are between 20,000 and 30,000 daltons.

"The challenge was how to make these huge molecules fly in the mass spectrometer. John Fenn, one of my science heroes who received part of the Nobel Prize for discovering electrospray mass spectrometry, tried putting it like this: 'Well, I gave molecular elephants wings'."

Spray-painting the proteins

Although Fenn got the very large protein molecules to fly, Carol Robinson wanted more – much more. She wanted to use the mass spectrometer to determine the very shape of the protein. She started to work on structural proteomics very early after the electrospray ionization technology became available. This enabled intact proteins to be introduced from solution to vacuum.

She got a crazy idea: if she could spray-paint the molecules, she could separate them based on how much paint they were covered by. The ultimate goal was to monitor protein-folding reactions by using mass spectrometry.

"Spray-painting an unfolded protein would take a lot of paint. But if it is folded into a very compact structure and then painted, I would not use so much paint, and that is exactly what we do. We spray it with deuterium, which weighs more than hydrogen. If an area of a protein is unfolded, hydrogen atoms are exposed and exchanged with deuterium. But if it is tightly folded, it won't, so it weighs less."

Carol Robinson developed a hydrogen-deuterium exchange method that could detect contact surfaces on proteins and applied it to study the folding of proteins. With this technique, protein structures could be preserved in vacuum, and this laid the basis for studying the structure of large proteins as well as protein–protein interactions using mass spectrometry.

A damning commentary

Carol Robinson used this technique to determine the folding state of a protein. However, her scientific colleagues thought she was crazy. At the molecular level, life as we know it occurs in water. A mass spectrometer has a vacuum.

"So it was really quite a damning commentary, and it was in a very respected journal: Proceedings of the National Academy of Sciences in the United States, so actually it was very hard to publish, because I would always have this cited when I submitted my research papers. People would say, 'Well, haven't you read this, it is a crazy idea.' I would say: 'Yes, I know, but I really believe it'."

In her quest to prove that mass spectrometers could be used to detect even large intact proteins, Carol Robinson had to break the existing mould of what was possible. This required developing a tandem mass spectrometer with improved ion transmission and a higher mass-to-charge range than before.

"At that time, the spectrometers typically took particles up to about 4000 mass-to-charge ratio. We thought we would be a bit revolutionary and go up to 32,000. That is a huge jump, and I remember people sort of cautioning me against that. They said just go to 8000. I said: 'No, 32,000 would allow us to do so much,' and I got one made and I bought it."

The new mass spectrometers proved Carol Robinson's point that protein complexes retain their structure in a vacuum; they also enabled researchers to detect intact protein complexes with a molecular mass above 500,000 daltons and to study subunit organization.

"Our experiments clearly established that protein complexes retain their subunit stoichiometry in the mass spectrometer. Today, these instruments are available from several mass spectrometry manufacturers and are widely applied in the pharmaceutical industry to investigate intact protein biopharmaceuticals, such as antibodies and membrane receptors, but at that time it was a small revolution."

A big breakthrough

Rather than just trying to show that they could do clever things with a mass spectrometer, Carol Robinson chose to take things to a new level.

"We thought maybe we can now answer some really key questions: for example, how the proteins come together in complexes. If we disrupted them, maybe they would fall apart in pairs, in threes or any other combination. And we could then tell from these interactions how they were assembled."

This new method enabled Carol Robinson and her colleagues to study how large protein complexes assemble and how proteins interact with co-factors and other proteins. In a pioneering work, she determined the conformation of GroEL, an important protein chaperone in the bacterium *Escherichia coli* that is highly conserved in humans.

"Chaperones protect other proteins while they are folding, creating a sort of protective environment. Our technique enabled us to examine its amazing structure. It has 14 copies of the same protein that form these two rings, and inside is the folding protein, and we started to look at how this protected environment would change folding."

Ever since then, Carol Robinson's group has studied even more important and complex structures such as binding interactions within an antibody–antigen complex, and this method is now used routinely for characterizing antibodies in the pharmaceutical industry.

"This has enabled more rapid characterization of antibodies and has advanced their use for treating cancer and many other diseases."

Giant soap bubbles

These methods have established Carol Robinson as a true pioneer in using mass spectrometry for analysing protein complexes. She has almost single-handedly founded a subfield of mass spectrometry proteomics, despite fierce criticism. This has required being fearless, innovative and creative. The latter became obvious when she decided to study some of the most challenging and important structures in biology – membrane proteins.

"Membrane proteins are incredibly hard to study, because one part of the protein exists inside an oily hydrophobic membrane, whereas the parts inside and outside of the cell are hydrophilic. We got the idea to coat them in detergent and then send them into the mass spectrometer in a giant soap bubble. And miraculously, this bubble shield really protects them, so they are released into the gas phase intact in a folded state."

In a series of landmark studies, Carol Robinson unravelled the structure of the proteins synthesizing our cell's energy currency, ATP, and how lipids play a key role in the structure and function of rotary ATPases, molecular motors involved in converting biological energy in our cells. Her more recent work on the role of lipids in membrane protein complexes has led her to study G protein–coupled receptors.

"These membrane proteins are targets for many drugs. We demonstrated that it was possible to maintain drug binding to G protein–coupled receptors and thereby identify natural ligands to these proteins. This enables new drugs to be identified that can bind to G protein–coupled receptors and thus target specific cellular processes."

Freedom of movement

Carol Robinson's group is now making exciting inroads into how fields related to mass spectrometry can dictate how drug discovery is performed. Once more, she has proved her critics wrong, who suggested that she had to use other techniques such as nuclear magnetic resonance or electron microscopy to study the structures of proteins.

"But if you think about a mass spectrometer, it is not in solution and it is not in a solid as a crystal would be, so it is not constrained. If you try to run through a swimming pool, it is really hard work. But if you want to express yourself, you want to be out in the air, so I think you could have your protein molecules in the gas phase rather than seeing it as a disadvantage."

Her critics claimed that protein folding in a vacuum is madness, but Carol Robinson sees it as an advantage, because the proteins maximize their freedom of movement. They can express themselves, and something can be learned from that movement. However, as always, scientists need to accept that, even though they find their ideas exciting, others might not.

"Sadly, if you do something for the first time, a lot of people do not believe it. So there was criticism of the first experiments, because people said: 'How can you measure folding in a mass spectrometer?' I always wanted to be able to do something for human health, and everybody would say to me: 'Well, that's really never going to happen,' but I think you just need to have the belief that it will."

So most importantly – according to Carol Robinson – you need to believe in your own ideas and follow your passion.

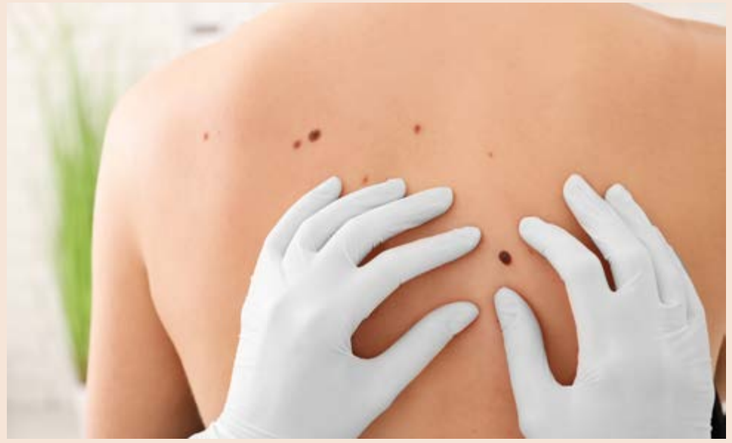
"I have had a great career in science, but I like to think that anyone can do this. I want to dispel the myth that you have to be a genius. You need imagination and creativity. Drive and energy. These are the most important things."

The 2019 Novozymes Prize was awarded at a ceremony on Friday, 15 March 2019 to Carol Robinson, Professor of Chemistry, University of Oxford.

Using laser-assisted drug delivery to combat major diseases

The skin provides vital protection against the external environment but also acts as a barrier for delivering medicine. Researchers have developed a technique that generates channels in the skin to enhance the penetration of topically applied drugs. Initial experiments in treating people with skin cancer are promising. Future perspectives for laser-assisted drug delivery are numerous and, for some diseases, the technique may prove to be more effective than injection or oral medication.

By Morten Busch



The key to curing people with disease is ensuring that medicine selectively reaches the cells affected by the disease. Traditionally, most medicine is given orally, which typically requires getting the active ingredient to cross the intestinal barrier or through injections and intravenous drip, which introduces the drugs directly into the tissue or bloodstream. Transdermal delivery avoids the pitfalls of pills, in which much of the active ingredient is lost as it crosses the intestinal barrier or is broken down by the liver. In addition, in treating people with a local disease such as skin cancer, the advantage of reducing the body's exposure to drugs and their side-effects is key. Researchers have therefore developed a laser technique that improves the delivery of medicine directly through the skin's surface.

"Drug penetration through intact skin is often limited by the size, charge and solubility of the molecules in a specific drug. In collaboration with the Wellman Center for Photomedicine at Massachusetts General Hospital in Boston, USA, we have shown that the delivery of anticancer agents into skin can be significantly enhanced. Ultimately, the techniques' applicability extends to a broad range of skin disorders and, potentially, systemic conditions," explains Emily Catherine Wenande, PhD student, Department of Dermatology and Copenhagen Wound Healing Center, Bispebjerg and Frederiksberg Hospital, Copenhagen.

Local combination chemotherapy

The researchers used skin from live pigs to examine whether chemotherapeutic (anticancer) agents can be delivered across the skin barrier. Light from an ablative fractional laser targets water in the skin, creating microscopic holes through the skin surface, thereby increasing its permeability.

"This technique creates hundreds of channels in the skin, enabling chemotherapeutic agents to reach their intended targets beneath the upper skin layers. Our group was interested in using affordable anticancer agents with proven efficacy against non-melanoma skin cancer. Two such agents, cisplatin and 5-FU (5-fluorouracil), are known to enhance each other's action when used in combination. However, they penetrate the skin barrier relatively poorly."

They hoped to take advantage of their combined effects by delivering both cisplatin and 5-FU directly into the skin using their laser-based delivery technique.

"When we delivered the two drugs in combination in live pigs, their inhibitory effects occurred more rapidly and were more pronounced. Within a couple of days, exposed skin sites developed wounds, and measurements of drug concentrations confirmed higher drug levels in laser-treated skin. These results were exactly what we hoped to see."

A new way to treat skin disease

After succeeding in combining the laser technique with the two chemotherapeutic agents, the researchers are now testing the treatment on people with basal cell carcinoma. This type of skin cancer is almost never fatal. However, it can be highly debilitating since people often develop multiple lesions, endure a high degree of recurrence and are commonly affected in delicate facial areas. The researchers hope that they can treat people with this type of skin cancer using the new treatment.

"Currently, basal cell carcinoma is either treated surgically, by radiation or, for more superficial lesions, using topical therapies. However, not everyone is a good candidate for surgery, and mutilating scarring and recurrence remain persistent challenges. Treating these people with cisplatin and 5-FU intravenously would never be justified since the disease is rarely fatal, but this new technique spares off-target healthy tissue, providing effective, local and less invasive treatment for people with skin cancer."

Since both cisplatin and 5-FU are already approved medications that have been commercially available for decades, the path to clinical implementation of the treatment is actually very short. However, the researchers believe that the techniques' potential goes far beyond treating skin cancer.

"In clinics across the globe, laser-assisted drug delivery is experimentally used for a broad range of indications, including delivering topical anaesthetics instead of by needle injection, as well as treatment of premalignant skin lesions, scars and vitiligo and for delivering aesthetic agents such as platelet-rich plasma, Botox® and vitamins. The technique is clearly quite versatile, and we are excited to play a part in continuing to refine it."

"Laser-assisted delivery enhances topical uptake of the anticancer agent cisplatin" has been published in Drug Delivery. In 2013, the Novo Nordisk Foundation awarded a grant to a main author, Merete Hædersdal, Clinical Professor, Department of Clinical Medicine, University of Copenhagen for the project New Targeted Treatment of Skin Cancer using Topical Administration of Anticancer Drugs in Combination with Laser-assisted Drug Delivery.

Drugs used to treat diabetes and obesity can reduce the urge to drink

Alcohol misuse is a massive social problem, causing 6% of deaths globally according to the World Health Organization. Pharmaceutical treatment of alcohol use disorder has scarcely changed in the past 25 years because developing and approving new treatments is both difficult and expensive. Researchers have now discovered that a drug already approved for treating diabetes also reduces the urge to drink alcohol.

By Morten Busch

Monkeys on Saint Kitts, an island in the Caribbean, are famous for voluntarily drinking large quantities of alcohol. This makes them ideal candidates for studying alcohol use disorder, which represents a major problem for people. Now Danish researchers have attempted to use a completely new and very surprising intervention to reduce the monkeys' desire to drink alcohol: glucagon-like peptide-1 (GLP-1) analogues, drugs that are normally used to treat diabetes and obesity.

"The monkeys' alcohol intake declined by 20–30% when they were given this drug. A possible cause may be that it inhibits the secretion of dopamine in the brain, resulting in the alcohol not providing the monkeys the same feelings of happiness as it would otherwise. Since GLP-1 analogues are already approved for treating people for diabetes and/or obesity, the path to an additional indication – alcohol use disorder – is actually shorter than if the drugs had not previously been used clinically," explains a main author, Anders Fink-Jensen, Consultant, Mental Health Services, Capital Region of Denmark and Clinical Professor, University of Copenhagen.

Affects the brain

Most monkeys on Saint Kitts are wild, but some are housed in groups in research facilities, and when alcohol consumption is studied, the monkeys have to be kept under controlled conditions to be able to measure their individual alcohol intake. Alcohol-preferring monkeys were offered alcohol for 4 hours every day for a 10-day period. The monkeys were then treated with two GLP-1 analogues, exenatide (Bydureon®) or liraglutide (Victoza®) for several weeks, and at the end of the treatment period their alcohol intake was measured for 10 days.

"Although we observed the same trends with both drugs, liraglutide clearly appeared to have the greatest effect, since it reduced the monkeys' alcohol intake by up to 30% compared with those that did not take the drug. Alcohol intake reverted to the normal level a few days after the monkeys stopped taking the drug. It is too early to conclude about any differences between the two drugs because the studies were not carried out simultaneously and because the alcohol intake differed in the studies."

These GLP-1 analogues have had similar effects in rats and mice, but this is the first time that the effects have been studied in primates, whose physiology is more similar to that of people. Some of the monkeys in Saint Kitts even have the same natural urge to consume alcohol as some people.

"The previous studies on mice showed that GLP-1 analogues influence blood glucose in the peripheral tissue, such as by GLP-1 analogues stimulating the release of insulin in the pancreas, but GLP-1 analogues probably influence alcohol intake by affecting the brain."

The researchers think that GLP-1 analogues affect the brain's secretion of dopamine, which means that people do not achieve the same feeling of happiness when they drink alcohol after being treated with these drugs. In a future clinical trial, the researchers will use brain scans to examine the possible central effects of GLP-1 analogues on the brain.

"We have already started a clinical study and expect to complete it in the next year. In addition to examining how GLP-1 analogues may affect the human brain, the research will also determine whether GLP-1 analogues also reduce alcohol intake among people with alcohol use disorder or excessive alcohol consumption."

A shorter path to treatment

These new results have wide-ranging perspectives. In Denmark alone, an estimated 160,000–180,000 people have alcohol use disorder. In addition, presumably 4–5 times as many people have excessive alcohol intake. About 20,000 people receive pharmaceutical treatment, with most taking disulfiram (Antabus®).

"The problem with Antabus® is that it does not eliminate the cause: the urge to drink alcohol. Of course, it gives people who are dependent a nasty reaction if they simultaneously drink alcohol, but the physiological craving for alcohol remains. We want to find a drug that can remove this craving."

Some research has been conducted on drugs that can eliminate the craving for alcohol, and several drugs in addition to disulfiram are registered for treating alcohol use disorder, but new and effective drugs are still needed to target this condition.

"The major benefit of GLP-1 analogues is that they are approved for treating people with diabetes and obesity, and since they are sometimes related to excessive consumption of alcohol, there is justified hope that people with diabetes who are overweight and have alcohol problems may benefit from GLP-1 analogues. People with normal weight who do not have type 2 diabetes may also benefit from this type of medicine. Future clinical trials must decide this."

"Effects of glucagon-like peptide 1 analogs on alcohol intake in alcohol-preferring vervet monkeys" has been published in Psychopharmacology. Researchers from the Novo Nordisk Foundation Center for Basic Metabolic Research participated in the study.

Small molecule can potentially combat many diseases

Many diseases and disorders are associated with inflammation in the body. Researchers have developed a small molecule that may counteract inflammatory conditions and can potentially be used for treating diseases ranging from heart disease and non-alcoholic fatty liver disease to stroke and lung infections.

By Kristian Sjøgren

When our bodies are exposed to oxidative stress, this affects the DNA, which begins to fragment. Luckily, the body has several mechanisms that can repair damaged DNA so it does not break down completely. However, while working hard to repair the DNA, these mechanisms can sometimes trigger inflammation in the body.

This type of inflammation, which can be chronic or temporary, is associated with many diseases. Examples include Crohn's disease, chronic inflammation of the gastrointestinal tract; non-alcoholic fatty liver disease; autoimmune diseases such as rheumatoid arthritis and psoriasis; cardiovascular diseases; osteoporosis; and some types of cancer.

However, good news may be on the way. New research shows that a tiny molecule (technically called a "small molecule" of about 1 nm) can inhibit one mechanism that contributes to inducing the inflammatory processes. This small molecule can potentially be used to combat all the diseases mentioned above.

"We have discovered a new function of an enzyme that has an important role in repairing DNA. When we suppress its activity, we inhibit the body's inflammatory response. This may be a very useful tool against many diseases involving an inflammatory response," explains a researcher behind the new study, Thomas Helleday, Professor, SciLifeLab, Karolinska Institutet, Sweden and Sheffield Cancer Centre, University of Sheffield, United Kingdom.

These results from Thomas Helleday and his colleagues at SciLifeLab have been published in *Science*. The research was carried out in collaboration between the University of Texas Medical Branch at Galveston, Stockholm University and Uppsala University.

Inflammation occurs when the body repairs itself

Thomas Helleday's research involves an enzyme called 8-oxoguanine DNA glycosylase (OGG1). OGG1 binds to and repairs DNA, and Thomas Helleday's research has shown that this promotes the development of inflammation. Among other things, OGG1-deficient mice are resistant to inflammation.

The specific mechanisms in the body's inflammation response involve the oxidized lesion of DNA, 7,8-dihydro-8-oxoguanine (8-oxoG) , accumulated in conditions of oxidative stress. 8-oxoG lesions are enriched in regions of DNA that are important for gene transcription, and binding of OGG1 to these regions will induce expression of inflammatory mediators and upregulate the immune response.

Thomas Helleday's therefore hypothesized that inhibiting OGG1 could inhibit the body's inflammation response in connection with oxidative stress.

"In principle, such a molecule can be used to treat many different conditions associated with chronic inflammation in the body. This also applies to obesity," says Thomas Helleday.

Cured mice with inflammation in the lungs

The researchers synthesized a small molecule, TH5487, that can bind to and inhibit OGG1 by changing its structure and thereby preventing OGG1 from attaching to and repairing DNA.

"This is proof of concept that our molecule functions as we intended. Now we need to investigate this in other virus-induced inflammation situations and in other parts of the body, but it looks promising."

Thomas Helleday

To test this molecule, the researchers induced acute lung inflammation in mice by giving them very large quantities of bacterial proteins or tumour necrosis factor-alpha (TNF-alpha), an inflammation-promoting substance naturally present in the body.

The type of lung inflammation the researchers induced in the mice sends the immune response into overdrive and ultimately causes the lungs to collapse because of damage to the alveoli. Half a million people die annually from this type of inflammation, and this also applied to the mice in Thomas Helleday's laboratory.

However, the experiments on mice showed that giving the mice TH5487 dampened the inflammation and reduced the damage to their lungs.

"This is proof of concept that our molecule functions as we intended. Now we need to investigate this in other virus-induced inflammation situations and in other parts of the body, but it looks promising," says Thomas Helleday.

May overtake TNF-alpha inhibitors as the world's best-selling medicine

Although taking newly developed concepts through clinical trials always requires a long time before any drug can be marketed, Thomas Helleday has hopes for his small molecule.

Drugs that inhibit TNF-alpha and thereby reduce inflammation in the body are the world's best-selling drugs and are used to combat rheumatoid arthritis, inflammatory bowel disease, psoriasis, asthma and many more diseases.

Thomas Helleday thinks that his molecule could potentially be used to combat even more diseases.

He again mentions lung inflammation, against which the molecule has only been tested in mice. Looking ahead, he imagines someone being admitted to a hospital with lung inflammation and being given a type of medicine similar to TH5487 that prevents the lungs from collapsing.

Thomas Helleday and his colleagues are working with such companies as Novo Nordisk on developing medicine of this type.

This molecule may also become useful in strokes.

A blood clot in the brain causes strokes. However, what often kills people is the oxidative stress and inflammation.

"OGG1 is involved in cleaving DNA, and this leads to inflammation. A stroke severely damages DNA, but cleaving all the DNA at the same time is not desirable because this results in an acute inflammatory response that is not good for the brain. Inhibiting OGG1 may therefore counteract some of the effects of a stroke," says Thomas Helleday.

"Small-molecule inhibitor of OGG1 suppresses proinflammatory gene expression and inflammation" has been published in Science. In 2017, the Novo Nordisk Foundation awarded a grant to Thomas Helleday, a principal investigator of the project "Progressing OGG1 inhibitors towards potential candidate drug for inflammation".

Technology leads the way

Content

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Researchers map the unique proteins secreted by cancer cells
Cancer cells in various tissues secrete various proteins. Researchers have now identified many of the proteins that cancer cells secrete. The discovery enables researchers to start creating diagnostic tools using a blood test that can identify almost any type of cancer.

Researchers vastly reduce the cost of cell experiments
The cost of experiments on cells in laboratories around the world can be vastly reduced based on a system researchers have developed for storing and working with cells during experiments. The system is one hundredth as large as today's standard system and therefore costs much less to operate.

Technology from the physics laboratory can make yoghurt more palatable
Scientists have used advanced laser technology to analyse the viscosity of dairy products. This technology can make it easier for food scientists and the food industry to analyse such properties as keeping dairy products from spoiling and optimally maintaining consumer interest.

Getting mould to make anticancer drugs
Most people want to avoid mould. These filamentous fungi can pose a real health hazard in water-damaged houses but they also produce several substances that can potentially combat diseases. Laboratory tests have shown that one such substance, calbistrin, can kill cancer cells. Now Danish researchers have found a way to get moulds to produce calbistrin in large quantities. This discovery can pave the way for future clinical trials with calbistrin and change how similar drugs are produced in the future.

DNA techniques unravel a prehistoric mass murder

Fifteen women, children and young men, each killed by blows to the head. Presumably in a massacre. But who were they? Many questions needed to be answered when archaeologists and genetic researchers collaborated in trying to understand the origins of a 5000-year-old mass grave in southern Poland. The results provide a unique perspective on a Europe affected by mass migration and violent conflicts.

By Morten Busch

Dramatic images are available of mass graves from recent massacres such as those in Bosnia and Herzegovina or Iraq. The bodies are often thrown into the graves like cattle – randomly and on top of each other. However, the mass grave archaeologists discovered near the village of Koszyce in southern Poland looked completely different. The skeletons were not randomly laid out. The 15 bodies had been carefully arranged, with some lying in pairs and some in groups. This intrigued the researchers so much that they decided to investigate who the 15 people were and why they were positioned as they were.

"We used radiocarbon dating and DNA techniques to unravel a family tragedy from the late Neolithic period. There is a mother with her children. A grandmother with her two sons. And two younger brothers together. The bodies were also flanked by grave goods, which indicates that those who buried the dead knew them well. The DNA also shows that the bodies were from Europe's Neolithic population and not the people from the Pontic steppe who were expanding westward into Europe at that time," explains a main author, Morten Allentoft, Associate Professor, Centre for GeoGenetics, Natural History Museum of Denmark, University of Copenhagen.

Three missing fathers

The bodies were buried 5000 years ago. Obtaining these exciting results would not have been possible a few years ago given the limited amount of DNA available because it degrades with time. This required the use of highly optimized ancient DNA technology combined with powerful next-generation sequencing machines to map the complete genome of each of the 15 skeletons.

"Previous studies have shown that we should try to take samples from the petrous bone inside the cranium. This is very hard, which means that the DNA is often extremely well preserved and not contaminated by other DNA such as that from soil bacteria. We extracted DNA from the right petrous temporal bone of all the skulls and successfully sequenced the genomes of the 15 skeletons in the grave."

The researchers combined modern gene-sequencing methods with archaeological methods, including carbon-14 dating, and determined not only when the 15 people had lived and how they died but also whether the people buried in the grave had been related.

"We concluded that they had been murdered, because they all had massive trauma to the head, but a real eye-opener was that the DNA analysis revealed that this was a large extended family comprising four nuclear families."

Although this was mass murder, the bodies had been buried very carefully, and painstaking genetic analysis enabled the researchers to determine exactly how the 15 people were related and how this influenced where they were positioned in the grave.

"They were not randomly positioned in the grave. An adult was next to a child. They turned out to be mother and daughter, and two young men lying next to each other turned out to be brothers. Everyone in the grave was a woman, a child or a young man. However, three fathers were missing. So our theory is that the fathers were absent at the time of the murders, discovered their murdered relatives when they returned to the settlement and then buried them."

DNA: our new history books

Besides the kinship among the 15 people in the grave, the researchers also determined the culture of these people. Archaeologically, they belong to the Globular Amphora culture that existed around 5000 years ago in Europe during the late Neolithic period. Genetically, they are also part of the typical Stone Age population in Europe.

"These murders occurred during a violent era in European prehistory. Five thousand years ago, the people of Europe experienced a reduction in Neolithic genomic ancestry as Yamnaya cultures migrated from the Pontic steppe. We demonstrated this in previous studies. But the DNA shows that the 15 people in the mass grave were Stone Age farmers with no steppe ancestry. Of course, we can never find out who killed them, but this extended family in Koszyce might very well have been killed as a result of territorial conflict during this dramatic time."

Niels Nørkjær Johannsen from the Department of Archaeology and Heritage Studies of Aarhus University came up with the idea of carrying out the DNA study of the mass grave in Poland. One goal was to reveal some of the prehistory of the European peoples, which was not particularly well known. The skeletons in the mass grave played a very special role in this.

"DNA analysis is helping to determine our prehistory, and typically we can only make general conclusions about the scale of population migration. But this grave reflects a specific event in a specific village and family and therefore provides us with a snapshot of an important and very violent time of transition in Europe," says Niels Nørkjær Johannsen.

"Unraveling ancestry, kinship, and violence in a Late Neolithic mass grave" has been published in Proceedings of the National Academy of Sciences of the United States of America. Co-author Simon Rasmussen is Associate Professor, Novo Nordisk Foundation Center for Protein Research, University of Copenhagen.

Researchers can finally control the viscosity of cell membranes

Researchers have discovered how they can very precisely control the viscosity of microorganisms' cell membranes. This means that they may be able to create custom cell membranes in the future. They can also improve the understanding of the causes of many diseases.

By Kristian Sjøgren

Cell membranes are the basis for all life, including humans, animals, plants and microorganisms. No cells could exist without cell membranes. Cell membranes confine the cell's DNA, prevent the nucleus from dissipating into the surroundings and exchange numerous molecules between the cell and its environment. This is equivalent to a Swiss army knife in biology.

Cell membranes comprise lipids (fat), wax, sterols (steroid alcohols), fat-soluble vitamins and much more. Researchers have now discovered how to very precisely manipulate cell membranes and control their viscosity, meaning how fluid and flexible the cell membranes are (low viscosity means more fluid). This discovery is revolutionary and paves the way for manipulating completely new forms of microorganisms with new properties and understanding various diseases.

"We now have many new opportunities to manipulate cell membranes and can study them more closely and, for example, make microorganisms more resistant to heat, cold or pressure. This has far-reaching perspectives in such fields as medicine and biochemical synthesis," explains the lead researcher of the new study, Jay D. Keasling, Professor of Chemical Engineering and Bioengineering and Principal Investigator, Keasling Lab, University of California, Berkeley and Lawrence Berkeley National Laboratory; and Scientific Director, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.

The study has been published in *Science*.

Cell membranes are dynamic

Although cell membranes may appear relatively static to the naked eye or under a microscope, they are not. Cell membranes are very dynamic, and their viscosity changes continually.

For example, when a cell needs to grow, the cell membrane must become very fluid so it can stretch and expand. When the cell environment is hot, the cell membranes must not be too fluid otherwise their whole structure collapses – similar to butter melting on a hot day. Conversely, cell membranes must not be too viscous when the cell environment is cold, because this makes the cells fragile.

Cell membranes have genetic and molecular tools that enable them to constantly adjust the composition of fatty acids in the membrane to control their viscosity. Unsaturated fatty acids make cell membranes more fluid, and saturated fatty acids make them more viscous. The ratio between the two types of fatty acids determines the viscosity.

Jay D. Keasling and his colleagues have discovered how to control this ratio between saturated and unsaturated fatty acids.

"Over the years, we have developed various molecular tools that enable us to very precisely control the ratio between saturated and unsaturated fatty acids in cell membranes. This means that, for example, we can now control whether a cell is able to grow and how much energy can pass through a membrane," explains Jay D. Keasling.

Manipulating E. coli bacteria to produce more unsaturated fatty acids

The tools the researchers use mostly comprise promoters that control the expression of genes that encode enzymes to produce each type of fatty acid. In their study, the researchers initially manipulated the genes of *Escherichia coli* bacteria and yeast cells to produce unsaturated fatty acids through an L-arabinose promoter. When the researchers gave the E. coli L-arabinose, a simple sugar, the bacteria increased their production of unsaturated fatty acids.

The researchers could thereby continually control the ratio between saturated and unsaturated fatty acids in the cell membranes of the E. coli. This is just one of the tools the researchers use.

"In the system we constructed, L-arabinose activates or stops the production of unsaturated fatty acids, like a light switch that is either on or off. In this analogy, we created a dimmer switch enabling us to make the light brighter or dimmer," says Jay D. Keasling.

Greater insight into diseases

Since the researchers can now control the viscosity of cell membranes, they will use the tools to both study and manipulate.

First, various diseases can be associated with changes in the viscosity of cell membranes. One such disease is diabetes; many researchers believe that a high-fat diet changes the composition of fatty acids in the cell membranes and thereby changes glucose uptake, insulin signalling and other factors. Huntington's disease is another disease researchers associate with changes in the viscosity of cell membranes.

The ability to manipulate the viscosity of cell membranes means that the researchers can more easily study how changes in viscosity may be associated with the development of various diseases.

This also applies to other major noncommunicable diseases.

"Cell membranes contain cholesterol, and manipulating cell membranes will enable us to study how we might be able to influence the high cholesterol levels that affect many people," says Jay D. Keasling.

"We can also imagine creating membranes of a specific viscosity that can purify water."

Jack Keasling

Producing bacteria for the chemical industry

Second, the researchers can also begin to manipulate new properties into microorganisms. One way is to change the viscosity of cell membranes to design microorganisms resistant to cold, heat or pressure.

Many companies use biochemical synthesis to make everything from chemicals to medicine. One problem is that the cells they want to use in the synthesis – often E. coli – cannot tolerate the optimal temperature or pressure for producing the desired substances.

"Our discovery brings us much closer to being able to produce cells that are custom-designed for chemical synthesis. We can also imagine creating membranes of a specific viscosity that can purify water. This discovery has many far-reaching perspectives," concludes Jay D. Keasling.

"Viscous control of cellular respiration by membrane lipid composition" has been published in Science. Jay D. Keasling is Professor of Chemical Engineering and Bioengineering and Principal Investigator, Keasling Lab, University of California, Berkeley and Lawrence Berkeley National Laboratory; and Scientific Director, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.

Whole-genome sequencing may revolutionize healthcare systems in Africa

For many years, researchers have subscribed to the dogma that bacteria cannot be whole-genome sequenced in Africa. Danish researchers have overturned this dogma and propose a new direction in healthcare that may significantly improve public health in African countries.

By Kristian Sjøgren

Imagine being infected by some exotic bacterium lurking mysteriously in your lunch. Fortunately, you live in Denmark, and a doctor can send a blood sample to a laboratory and find out a few days later which bacterium has caught you. Then the doctor just chooses the right treatment to make you well again.

Then imagine living in a small town in the middle of Africa. The situation is very different, because no one has either the knowledge or the equipment to determine which bacterium has infected you. Finding out whether you have a Salmonella infection, for example, can take several weeks. For local inhabitants in Africa, waiting can be painful and in some cases fatal.

However, good news is on the way. Danish researchers are the first globally to show that minimal investment in a single machine can replace equipment worth millions and decades of expert knowledge. The machine can do everything in much less than half the time. This works in Denmark and can also work in Africa.

"We have shown that healthcare professionals in Africa can be trained to use a whole-genome sequencer costing 500,000 kroner. This means that the waiting time to identify a pathogenic bacterium can be cut from a couple of weeks to 48 hours. This can massively improve public health in Africa," explains the researcher behind the new study, Frank Møller Aarestrup, Professor, National Food Institute, Technical University of Denmark.

He and his colleagues have published their research results in the *European Journal of Clinical Microbiology & Infectious Diseases.*

Advanced equipment can definitely be used in Africa

Understanding the new results is not especially complicated. The researchers installed a whole-genome sequencer in a hospital in Moshi, Tanzania and trained healthcare professionals to use and service it.

The aim was to see whether the personnel available could operate the sequencer and get it to function under local conditions.

They could. No ifs, ands or buts!

The sequencer enabled local healthcare professionals to identify the bacteria from infected people in just 48 hours, much more rapidly than previously.

"It may sound easy, but many researchers did not believe that this could be done in Africa, which in many ways still has very underdeveloped healthcare systems. I often meet researchers who condescendingly do not think that local personnel in Africa can administer this type of machine over the long term, but they can," explains Frank Møller Aarestrup.

Denmark is still using old equipment

High-income countries differ enormously from low- and middle-income countries in the normal procedures for identifying harmful bacteria.

A country like Denmark has accumulated a well-functioning arsenal of laboratory equipment over decades, enabling bacteria to be identified in many different ways. Traditionally, Denmark has used the Petri dish, growth media, microscopes and expert knowledge, but today whole-genome sequencers are used to perform most of the analysis based on the DNA from a bacterium. The results can then be used to determine the species.

Low- and middle-income countries that do not have access to laboratory equipment costing billions have two options:

- invest in the traditional equipment and develop the necessary expertise; or
- invest in new equipment.

"Conservatism is rife in this field, and even in Denmark we still use some old equipment because we have a tradition of doing so. However, I do not think that countries in Africa, for example, should take this path. Instead, these countries should use the new methods and learn to operate the equipment correctly," says Frank Møller Aarestrup.

Database matches DNA against known bacteria species

Whole-genome sequencers have many advantages.

- The sequencer functions very simply. DNA is extracted from a bacterium and fed into the sequencer, which then spits out a long DNA sequence of about 7 billion letters. This information is unusable in its raw form, but comparing it with a database containing the DNA sequences for all known bacteria immediately reveals the identity of the bacterium being tested.
- In addition, the sequencer can detect small differences between individual bacteria, enabling healthcare personnel to determine whether the bacterium infecting you is the same as that infecting your neighbour. This enables researchers to follow the transmission of the infection and identify the source.
- Doctors can also match bacteria with known types of multidrug-resistant bacteria enabling them to prescribe the right type of antibiotics.

"Whole-genome sequencing enables hospitals to diagnose the cause of an infection much more rapidly and precisely. This can significantly reduce the treatment time in places where this currently takes a long time," explains Frank Møller Aarestrup.

New knowledge causes new headaches

Nevertheless, not everything in Moshi is perfect.

Doctors can now use a whole-genome sequencer to determine that many infections spread within hospitals, and this obligates them to act. But they do not know how.

"The problem is that doctors now have some information that they would rather not have, because they do not know how to stop transmission. However, the research project is not focusing on this now, but this is a separate problem that needs to be addressed," says Frank Møller Aarestrup.

Danish Veterinary and Food Administration uses only sequencing

Previously, Frank Møller Aarestrup's research group conducted another study showing the power of whole-genome sequencing in identifying infectious bacteria.

Some years ago, they evaluated the potential of whole-genome sequencers for identifying foodborne bacteria. The study compared the performance of a sequencer with the performance of traditional methods; the sequencer identified a bacterium a whole week sooner than the traditional methods and was even cheaper.

"The result was that the Danish Veterinary and Food Administration uses only sequencing to identify bacteria today. Hospitals in Africa should also do this, and we have shown that this is feasible," concludes Frank Møller Aarestrup.

"Molecular epidemiology of virulence and antimicrobial resistance determinants in Klebsiella pneumoniae from hospitalised patients in Kilimanjaro, Tanzania" has been published in the European Journal of Clinical Microbiology & Infectious Diseases. In 2016, the Novo Nordisk Foundation awarded a Challenge Programme grant of DKK 60 million to Frank Møller Aarestrup for the project Global Surveillance of Antimicrobial Resistance.

In the future, we will use living medicine

Microorganisms do not always make us ill. The correct composition of bacteria in our intestines can make us healthier and even heal illness. Scientists will now programme bacteria in our intestines to produce medicines that can make us healthy when we are sick. If this succeeds, it will enable bacteria to be used to combat most diseases and replace traditional pill-based treatments.

By Morten Busch

Ever since penicillin was discovered in 1928, humanity has used the arsenal of microorganisms to combat other microorganisms that cause disease. Researchers have screened fungi and bacteria for substances that kill or inhibit the growth of pathogenic bacteria and then extract them and use them as effective antibiotics. With current medicine, dosing is a big challenge in effectively defeating infection. A major Danish research initiative aims to solve this problem.

"People typically take one or a few doses a day, so the concentration of medicine in the body varies greatly over time. Instead of this traditional treatment, we will use specially engineered bacteria that can produce the pharmaceutically active molecules on demand. We hope that this living medicine can lead to more effectively treating people with disease in general," explains Morten Sommer, Professor, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.

Bacteria react as the human body would

The new technology, advanced microbiome therapeutics, works by modifying the genome of bacteria so they secrete specific molecules that can influence other bacteria or the body's mechanisms. Traditionally, medicine has been given orally, intravenously or topically. However, the body's systems often eliminate the medicines rapidly. Regular dosing is therefore necessary to maintain treatment.

"Advanced microbiome therapeutics provides a unique opportunity to change this model. When bacteria colonize the intestines, they create a stable version of the therapeutic compound, and the aim is to get them to respond to their surroundings so they can secrete the required compound on demand. We will thus be able to treat diseases in a precise and targeted manner."

"The idea behind living medicine is to treat a disease in the same way as the body would do it."

Morten Sommer

Nevertheless, the idea of getting microorganisms to combat other microorganisms in the body is far from new. For years, research on the human microbiome has focused on the importance of having healthy intestinal flora. In the quest to create healthier intestinal flora, researchers have previously experimented with probiotics in milk products or, in more extreme cases, transplanting intestinal tissue from donors with healthy intestinal flora.

"The idea behind living medicine is to treat a disease in the same way as the body would do it. If the body would respond to a disease, such as diabetes, it would produce insulin in response to certain signals in the body, and that would lower the blood sugar. We can achieve a similar thing with bacteria because we can engineer them to respond to the same signals and produce those same molecules as the body."

Communication is the most difficult

Living medicine can therefore potentially treat a variety of diseases. By modifying the bacteria, the researchers can even tailor them to solve the health challenges of individual people. The researchers can also ensure that the bacteria persist only in one individual's intestines and cannot spread to other people.

"Ensuring that cell-based medicine cannot spread in an uncontrolled way between people is central to the project. Over the past years, we have learned how to engineer very sophisticated control systems in bacteria, and that allows us in a variety of different ways to ensure that a bacterium persists only in the patient who is treated and only for the time period that we would like them to persist for."

However, the greatest challenge in developing living medicines lies elsewhere: in how the many complex bacterial communities interact in our body.

"Currently, there is relatively little information about how engineered bacteria will interact with a bigger community of bacteria in our intestinal system. That is going to be a major thing we will study in this project. We will aim to develop strategies that allow us to override some of these interactions to ensure that the living medicine has an opportunity to establish itself."

Of course, the researchers dream of developing new treatments for specific life-threatening diseases.

"A successful outcome of this project would be developing, in 6 years, a framework for how we can engineer living medicine, how we can control the bacteria and how we can use bacterial therapy to treat people."

In 2018, the Novo Nordisk Foundation awarded a Challenge Programme grant to Morten Sommer, Professor, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, for the project Design and Engineering of Biological Molecules and Systems. The project will be carried out in collaboration with Fredrik Bäckhed, Professor, University of Gothenburg; Max Last Nieuwdorp, Professor, University of Amsterdam; and Tine Rask Licht, Professor, Technical University of Denmark.

Researchers produce cannabinoids by using yeast

The market for cannabis has taken off in recent years, and it will definitely grow in the future. Researchers have finally discovered how they can synthesize the relevant cannabinoid compounds in cannabis by using yeast, opening up new pharmaceutical applications.

By Kristian Sjøgren

The cannabis plant contains more than 100 cannabinoids; the two most commonly known are tetrahydrocannabinol (THC) and cannabidiol (CBD).

More and more countries are now legalizing cannabis products. In Denmark, certain cannabinoid formulations have also been approved for use, which enables people to obtain prescriptions for, among other things, tea with cannabis or CBD oil.

Cannabinoids have both pharmaceutical and relaxing effects on humans. People with epilepsy or multiple sclerosis, for example, often benefit from treatments with cannabis products, and skin care products and coffee containing cannabinoids are now available. Breweries are also starting to examine the possibility of making cannabinoid beer, and soft drink manufacturers want to make sports drinks containing cannabinoids.

The need for controlled synthesis of large amounts of cannabinoids is therefore rapidly growing, and researchers have now succeeded in getting yeast to produce them for the first time. This opens up even more potential applications of cannabis.

"Cannabinoids are both expensive to produce synthetically and bad for the environment, and producing them through traditional cannabis cultivation is still illegal in many countries. A different production method is therefore required, and yeast is an obvious candidate. We can now produce cannabinoids through an ordinary fermentation process, similar to making beer," explains the researcher behind the cannabinoid yeast, Jay Keasling, Professor, Keasling Lab, University of California, Berkeley and Scientific Director, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.

The spectacular results were recently published in *Nature*.

Cannabinoids are already being used pharmaceutically

THC is the most psychoactive substance in the cannabis plant and is currently used pharmaceutically for treating such ailments as nausea and vomiting in connection with chemotherapy and anorexia and to counteract weight loss among people living with HIV.

CBD is used to treat epilepsy and other disorders of the nervous system.

Researchers know little about the properties of the many other cannabinoids in cannabis. However, they need to isolate them individually to carry out experiments.

The current price of synthesizing cannabinoids is USD 40,000–70,000 per kg. The price for making them with Jay Keasling's yeast will be USD 400.

Producing cannabinoids using yeast also costs only 10% of the cost of growing cannabis in a greenhouse, and price is not the only advantage.

"Cultivating cannabis is both expensive and bad for the environment. Growing cannabis requires an estimated 3% of all electricity used in California, and the production process also uses large amounts of water. In addition, producing cannabis is very wasteful because only the plant shoots are used. Production using yeast creates none of these problems," says Jay Keasling.

Synthesizing unnatural cannabinoids with yeast

In addition to producing the pure cannabinoids for a fraction of the price, the newly developed cannabinoid yeast also has a third advantage: the researchers can create completely new forms of unnatural cannabinoid analogues.

Adding various fatty acids to the yeast produces various cannabinoids the natural plant cannot produce. According to Jay Keasling, some of these may therefore be developed into pharmaceutical breakthroughs.

Among other things, various chemical changes in the cannabinoids might make them more potent, provide them with other effects or enable them to bind to various types of medicine, thereby creating new types of combination treatment.

"The yeast is a little imprecise in producing the various cannabinoids, and this means that it can produce some cannabinoid analogues that the plant cannot. This is very beneficial for discovering cannabinoids with either new or more potent properties," says Jay Keasling.

Jay Keasling and the University of California, Berkeley have patented the cannabinoid yeast and launched Demetrix, a company that aims to develop new types of medicine based on the opportunities opened up by cannabinoid yeast.

"We can already synthesize exactly what people need. Right now, we are focusing on the natural and commonest types of cannabinoids, but later on, we plan to move to the rare and unnatural types. We will definitely collaborate with the pharmaceutical industry and the University in testing various cannabinoids to treat various diseases," says Jay Keasling.

Splicing genes from other organisms into yeast

The researchers took five genes from the cannabis plant and from other organisms and spliced them into the yeast cells.

The genes encode various steps in the process that eventually results in the production of cannabinoids.

The researchers selected some genes from other organisms in places where the genes are more efficient than the cannabis genes in making the basic molecules that later become cannabinoids. They then used specialized genes from the cannabis plant to make the final cannabinoids.

"The genes encode various enzymes, and we chose the most efficient ones available," explains Jay Keasling.

Researchers racing to develop cannabinoid yeast

Jay Keasling's research team was not the only one striving to get yeast to produce cannabinoids.

The market for cannabis products is growing tremendously, so every patent is a potential gold mine.

The race between the various research groups trying to do what Jay Keasling ended up doing first was therefore intense.

Many of the other researchers got stuck when they tried to find an enzyme that creates the parent molecule from which all cannabinoids are formed.

Canadian scientists had already patented the discovery of an enzyme they believed was responsible for the process.

However, no matter how hard Jay Keasling and his research team tried to splice the gene for that enzyme into their yeast, they could not make it work. This made them go back to the cannabis plant and search again.

They found another gene in the plant, spliced it into their yeast, and it began to produce huge quantities of cannabinoids.

"This problem was so great that it became a barrier for many of the other research groups, and we ended up winning the race," says Jay Keasling.

"Complete biosynthesis of cannabinoids and their unnatural analogues in yeast" has been published in Nature. Jay Keasling is Professor of Chemical Engineering and Bioengineering and Principal Investigator, Keasling Lab, University of California, Berkeley and Lawrence Berkeley National Laboratory and Scientific Director, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.

Scientists use evolution to revive zombie genes

Bacteria have many pseudogenes that have lost their function, but researchers have learned how to use adaptive laboratory evolution to revive them. This discovery opens up great pharmaceutical possibilities.

By Kristian Sjøgren

The Technical University of Denmark has developed a method that speeds up evolution by pressuring bacteria and fungi to develop at a furious pace.

Researchers have now discovered that this method – adaptive laboratory evolution – can also revive pseudogenes.

The discovery is interesting because it indicates why evolution has retained these zombie genes that could otherwise easily have been discarded. The pseudogenes probably function like spare parts that keep an old car working so it can be used when the new one has broken down.

"This is fascinating. For the first time, we can show that pseudogenes can be repaired and probably have an evolutionary function we had not known about," says a researcher behind the study, Bernhard Palsson, CEO, Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark.

The new study was recently published in *Nature Microbiology*.

Pseudogenes are a legacy of the past

Understanding the new findings requires knowing what a pseudogene is.

A pseudogene is a gene that no longer functions and has shared ancestry with a functioning gene. For example, in evolutionary terms a bacterium may no longer require a specific protein coded by a specific gene and because this protein is not required for the organism to survive, the gene does not need to be repaired.

The pseudogenes are a legacy in the genome from a time when the bacteria functioned differently. Bacteria have up to 10,000 genes and also between 100 and 1000 pseudogenes that have no function and appear not to have any purpose.

The pseudogenes might be expected to disappear over time, but bacteria seem to retain them, which has long puzzled researchers.

They finally have an idea why the bacteria do not discard the pseudogenes.

Bacteria find new ways to absorb iron

The researchers investigated what happens to *Escherichia coli* if they gradually remove parts of the genes the bacterium uses to make proteins that draw iron into the bacterial cells.

When bacteria enter a host, they need iron to divide and proliferate, and they absorb the iron from their surroundings using specific proteins. When these proteins no longer function, the bacteria stop growing and dividing.

The researchers observed the same process in their evolution-accelerating process when they removed the genes from the bacteria. As expected, the bacteria stopped growing.

However, the researchers were also surprised to see that one of the bacterial cultures suddenly began to grow again, as if it still had fully functional versions of the genes the researchers had removed.

The researchers analysed the genome of the bacteria and discovered that the bacteria still lacked the gene that had been removed. Instead the bacteria had repaired a pseudogene, which caused the bacteria to produce a different protein that could enable the cells to absorb iron.

Discovered several repaired pseudogenes

Based on numerous experiments, the researchers at the Technical University of Denmark examined the DNA sequences of 300,000 bacterial genomes in which bacteria have adapted to survive in ways for which they were not really designed.

"The bacteria repaired a pseudogene that they were not using anymore. It was a minor repair. The bacteria needed to either remove two nucleotides from the DNA or insert four to activate the gene. The interesting thing is that they do this when we put the bacteria under evolutionary pressure during the evolution-accelerating process," says Bernhard Palsson.

Forcing bacteria to evolve

The researchers have used adaptive laboratory evolution for some years. This enables them to apply strong selection pressure to bacteria by, for example, removing the type of sugar they normally metabolize. The bacteria then have to adapt to metabolize something else and the evolutionary process has to accelerate to achieve this

For example, some bacteria prefer to metabolize glucose, but if pressured because of the lack of their preferred food, they can develop the genetic basis to enable them to grow on other types of sugar.

This is like an evolutionary process providing people with the metabolic enzymes and proteins to enable them to live by eating grass or bark.

"Adaptive laboratory evolution enables us to observe evolution in action," explains Bernhard Palsson.

One explanation may be that the researchers made them grow on new types of substrates, forced them to survive in various toxic chemicals or destroyed parts of their metabolism genetically.

If possible, the bacteria develop what they need to survive, and the researchers can genetically examine how they have done this.

After the researchers discovered the repaired pseudogenes, they returned to their database of 300,000 bacterial genomes to see whether any of the bacteria with which they previously had worked had also recreated lost functions by repairing pseudogenes. Here they found several other examples of bacteria that had taken old genes into use to survive.

"This provides fascinating insight into how evolution works. Organisms do not seem to discard pseudogenes because they provide opportunities to survive. This is a genetic reservoir that they can use if needed. This discovery is really interesting," says Bernhard Palsson.

Can be used commercially

The insight into the evolutionary engine room and the development of adaptive laboratory evolution open up various commercial opportunities.

Researchers can use the method to force bacteria to produce things they would not normally. These could include various products of pharmaceutical interest, such as adrenaline, dopamine or melatonin, all of which are hormones in humans.

Researchers who know the evolutionary mechanisms of the bacteria can link a gene that produces one of these hormones to the metabolism of the bacteria, and although the bacteria do not normally produce the hormone, they are forced to do this to survive.

Further, the bacteria have to find evolutionary solutions to enable them to survive when producing the hormones. In this respect, nature and evolution are far better at finding solutions than researchers are.

"We can harness evolution to get bacteria to develop various molecules for us in many different ways. Adaptive laboratory evolution has developed from being a tool to study bacteria and fungi to becoming a tool to specifically design substances with the help of evolution," says Bernhard Palsson.

"Pseudogene repair driven by selection pressure applied in experimental evolution" has been published in Nature Microbiology. Several co-authors are employed by the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Lyngby.

Amazing connections

Content

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Inpatients' health suffers if they sleep poorly
Anxiety, light, physical inactivity, noise and many other factors can cause patients to sleep poorly in hospitals. This negatively affects patients' immune systems and recovery processes, which can result in longer hospital stays.

Premature birth can cause heart problems later in life
The part of the nervous system that regulates the heart rate and other autonomic functions develops in late pregnancy. Now new research shows that premature birth may weaken this regulation. This may also explain why individuals born preterm may have an increased risk of cardiovascular disease later in life.

Researchers search for the tremors that stop the heart
Cardiovascular diseases cause more deaths than any other in Denmark. Narrowing of the coronary artery can lead to a blood clot blocking the blood flowing to the heart. Balloon angioplasty saves many people's lives, but 1 in 10 have fibrillation inside the heart – often resulting in death. Researchers are now finding early electrical irregularities that can predict heart fibrillation before it occurs.

People with psoriasis develop cardiovascular disease more frequently
Compared with the major global diseases, psoriasis is easily overlooked, but it affects people both physically and mentally. New research shows that psoriasis does not only affect the skin. People with psoriasis develop cardiovascular disease much more frequently. The reason remains unclear, but the researchers believe that doctors should pay extra attention to the diseases that may result from psoriasis when treating the people who have it.

Researchers discover genetic link to impaired language among children with autism

An international research group has discovered an association between how genes are expressed in blood and impaired language development among children with autism. The discovery may pave the way for a blood test that can diagnose children with autism at birth.

"Large-scale associations between the leukocyte transcriptome and BOLD responses to speech differ in autism early language outcome subtypes" has been published in Nature Neuroscience. Nathan Lewis is Associate Professor, Systems Biology and Cell Engineering and Novo Nordisk Foundation Center for Biosustainability, University of California, San Diego, USA.

By Kristian Sjøgren

Autism clearly has a genetic and hereditary component, and researchers now understand this better after studying immune cells in the blood.

More precisely, researchers from the United States, Cyprus and elsewhere have discovered that they can use the gene coexpression in white blood cells to determine whether children with autism have genetic traits that imply impaired language development related to autism spectrum disorder.

In the long term, this discovery may assist in developing a blood test that will enable doctors and parents to determine whether a child might, or might not have autism – at a much earlier stage than today.

"Today, autism is diagnosed based on mental characteristics. However, the tests are usually not effective until a child is older than 3 years. Knowing the hereditary component of autism will enable us to identify this in a blood sample from children so that we can diagnose them and intervene with supportive treatment much earlier than we can today," explains a researcher that participated in the new study, Nathan Lewis, Associate Professor of Pediatrics and Bioengineering, University of California, San Diego, USA.

The study was recently published in *Nature Neuroscience*.

Researchers cannot identify autism genetically in most cases

The hereditary nature of autism is undeniable and accounts for 80% of the risk of developing it. Nevertheless, researchers have extreme difficulty in identifying autism by examining genes. Hundreds of genetic mutations and variants have been identified that predispose a person to developing autism. However, each of these can explain less than 1% of the risk of developing autism.

Even when researchers combine all these small differences, they cannot explain more than 5–10% of the risk of developing autism.

Evidence therefore indicates that the hereditary component of autism cannot be identified through differences in the genes for many people, but rather in differences in how the genes are expressed, or in a cocktail effect, in which combinations of commonly occurring genetic variants make people more likely to develop autism than they would based on the sum of the individual genetic variants.

"Epigenetics may cause the development of autism. This means that we cannot examine the genes in isolation to determine whether one child will develop autism while another will not. Their DNA will be similar, but the way the body's cells decode the DNA differs, and that may cause autism," explains Nathan Lewis.

Only some children with autism develop language late

This situation is further complicated by the fact that autism is a spectrum disorder, which means that it takes many different forms and probably originates from many different neurobiological backgrounds.

Some children with autism are intellectually impaired; others are genuinely brilliant. Some are very sensitive to food; others are not. Some develop language very late in childhood; others develop language normally.

One great challenge in understanding the neurobiological background of autism is to understand its different manifestations.

In this study, researchers from San Diego and Cyprus investigated differences in gene coexpression in the blood among children with autism and late language development versus normal language development.

Many children with autism develop language very late, which is a sign psychologists use to diagnose these children. For other children with autism, their language development does not indicate whether they have autism.

"Our goal was to look for groups of genes that collectively express themselves differently between the two groups. These genes may help explain the changes in the brain among children with autism who have late language development," says Nathan Lewis.

Finding signs of autism in the blood

The researchers collected blood samples from 118 infants with autism. Then they examined how the white blood cells expressed messenger RNA (mRNA), which is a marker for the overall activity of the genes and contains results from genetic mutations and variants, epigenetics and so on.

The researchers examined white blood cells because they are constantly being recreated. This means that the same steps that occur as the fetus develops, including the brain, are continually repeated in the white blood cells. This can provide researchers a window into the time when things may go wrong during fetal development and the foundations of autism are laid.

"Epigenetics may cause the development of autism."

Nathan Lewis

The results showed a clear difference in gene coexpression among children with autism with normal language development versus late language development. One group of children expressed several types of mRNA that were not expressed to the same extent in the other group.

The researchers examined the genes closely, and many were specific to humans and linked to language development. The genes are expressed in many different tissues, including the brain and the white blood cells, and have also been identified as influencing the development of autism.

This is interesting. Many of these genes play a role in brain development in the first trimester of pregnancy. Our findings show neurobiological differences among children with autism who have late language development versus those who do not and that these differences are already present before the children develop their language difficulties," explains Nathan Lewis.

Discovery can be used for diagnosing autism

The new results give researchers greater insight into autism and its various subtypes. In addition, the results provide insight into developing diagnostic tests that can determine whether a child has autism or not.

Language problems are an early sign of the development of autism, and the fact that researchers can show a measurable neurobiological association with language difficulties enables language support and other actions to be implemented much earlier to help the child develop as normally as possible.

"Although we may not be able to find specific genetic mutations to explain the development of autism, we can pinpoint the genes that are involved and expressed in changes in brain development in the early fetal development stage among children with autism. We have finally begun to understand the neurobiological background for the development of autism. This knowledge will likely make it possible to more effectively treat people with autism in the future," says Nathan Lewis.

Molecular trap can help young children avoid painful bone surgery

A single mutation of one gene can greatly influence a person's life. One such mutation causes achondroplasia, characterized by disproportionate short stature, reduced space for the brain and debilitating respiratory complications. Currently, it has only been possible to treat the symptoms by limb lengthening, an extremely painful surgical procedure. Now scientists have discovered a molecular trap that can capture the proteins that cause the disease. This treatment is already on the way in a Phase 1 trial.

By Morten Busch

Growth is normally good, but not if it is unimpeded. This is why the human body is equipped with mechanisms that can inhibit growth. About 1 in 25,000 children have a genetic mutation in this inhibition mechanism at birth, resulting in the person being considerably shorter than average. What's worse is that infants with achondroplasia have life-altering complications because the cranium has insufficient space for the brain and other body systems do not develop in a proportionate way, causing, for example, breathing problems. Today, only the symptoms can be treated. However, in the near future the cause of the disease can hopefully be treated.

"These children are affected because the mechanisms that help to inhibit their growth are constantly active. This new research has shown the promise that trapping the growth-inhibiting signalling protein with the protein TA-46 and treating children from infancy can normalize their growth so they can avoid complications in the brain, spine and airways," explains Luca Santarelli, Therachon's Chief Executive Officer, who is responsible for developing and testing this new treatment.

Life-threatening brain condition

One gene mutation in the FGFR3 gene, which codes for fibroblast growth factor receptor 3 (FGFR3), causes 97% of achondroplasia cases. For most people, fibroblast growth factor 3 (FGF3) binds to the receptor on the cell surface, sending a signal to inhibit growth. Once the signal has been sent, FGF3 is absorbed and broken down in the cell. However, people with achondroplasia have a mutation in the receptor that results in FGF3 not being absorbed and broken down.

"These children's bone growth is therefore constantly inhibited, and the only known treatment is limb lengthening, an extremely painful and invasive surgical procedure to make them taller. We hope that the new drug will be able to treat the actual cause of the disease so that children can fully or partly avoid these potentially lifelong symptoms."

Short stature is the most visible sign of achondroplasia. Men with achondroplasia average only 131 cm in height and women 123 cm. Being shorter and having shorter arms can make daily life challenging for everything from driving to shopping. Even worse, however, are the serious symptoms affecting infants with achondroplasia.

"One life-threatening condition is an abnormality of the junction between the cranium and the spine that reduces the space for the lower brain stem. This can result in death, and we therefore hope that our treatment can ensure that children grow normally from infancy," explains Aled Williams who is Chief Commercial Officer in Therachon.

A eureka moment

The idea for the new treatment dates back to 2013, when Elvire Gouze, Stéphanie Garcia and their research colleagues from the Mediterranean Centre for Molecular Medicine in Nice, France noticed that people with bladder cancer also had these problems with the FGFR3 receptor. These researchers had some success using antibodies against FGF3 but had an even more brilliant idea.

"They produced a soluble form of the FGFR3 receptor that could bind to FGF3 and 'trap' it to prevent it from binding to the cells' receptors. Treating mice with the liquid receptor reduced the growth-inhibiting signals so that the bones of the mice developed almost normally. This was a eureka moment, because the results were so clear."

The soluble FGFR3 receptor traps the surplus FGF3 molecules, solving the problem that the receptors on the cell surface do not function optimally. The research results were so convincing and unequivocal that investors were eager to convert the research into an actual treatment. Therachon came into the picture at that point.

"It's rare to find a disease in which the cause and treatment are so obvious and easily quantifiable. In addition, medicine has previously been used to trap problematic molecules so this presented no problems. Further, the initial trials showed no noticeable side-effects."

Early treatment possible

Although the results and methods are simple, there are the usual major challenges associated with drug development and the path to a marketable medicine. The French prototype was especially challenging because it was unstable. The researchers therefore had to work on developing new and more stable versions of the soluble receptor.

"In recent years, we have further developed the drug into a version that is stable both when stored at hospitals and when injected into the human body. In early 2016, we were finally satisfied with the TA-46 candidate with which we had been working, and in early 2018 we began a Phase 1 trial with healthy participants to assess the safety, side-effects and dosage."

It is still too early to predict when the testing of this new treatment will be complete because this involves both Phase 2 and Phase 3 trials. However, the researchers hope to be able to offer treatment as part of the clinical trial programme to people with achondroplasia within the next year or so. The initial plan is to inject the medicine into children and adolescents with achondroplasia once a week.

"Increasingly, however, children can be diagnosed through prenatal ultrasound scanning. So in time, it may be possible to treat children very early in their life, thereby avoiding the complications and greater risk of death in the first years of their lives."

"Postnatal soluble FGFR3 therapy rescues achondroplasia symptoms and restores bone growth in mice" has been published in Science Translational Medicine. In August 2018, Novo Holdings and co-investors Cowen Healthcare Investments, Pfizer Ventures and funds managed by Tekla Capital Management LLC invested USD 60 million in Therachon's ongoing development of a treatment for achondroplasia.

Waterproofing and non-stick pans may lead to earlier puberty among girls

Waterproof raincoats and non-stick pans make our everyday lives more practical. Unfortunately, some of the perfluoroalkyl substances that provide the practical benefits end up in the water and air and may harm our health. A new study appears to show that these substances are associated with girls starting puberty prematurely. Boys also seem to be affected by the substances. The researchers hope that manufacturers will improve at investigating the potential long-term effects before using such substances.

By Morten Busch

Children start puberty today much earlier than they did 150 years ago. Better living standards have caused much of this, but even though they have not changed significantly in recent years, research shows that the age at which puberty starts has continued to decline. A Danish research project has now shown that the perfluoroalkyl substances in our environment may play a role in this trend. The substances are in our environment because they are used in many things we use every day: packaging, blankets, jackets and pans.

"These substances are present in the air, water and dust that surround us. We measured the concentration of these substances in pregnant women's blood and showed that the children of the women who have the highest concentrations start puberty earlier, and this may affect their health in the long term. The changes seem to apply to both the first generation of these substances but also to the new generations," explains the study's first author, Andreas Ernst, PhD student, Department of Public Health, Aarhus University.

New substances have the same effect as the old ones

The study is part of the project Better Health in Generations, which collected data from 92,000 pregnant women in 1996–2003. Since then, the children resulting from these pregnancies have been regularly followed up with questionnaires. Since 2012, 22,341 of the children born in 2000–2003 have been invited every 6 months to answer questions about their pubertal development, such as whether they had begun to develop breasts and pubic hair.

"We therefore had a unique opportunity to compare the children's responses with the concentration of the perfluoroalkyl substances in their mother's blood samples while they were pregnant, and this indicated a rather clear association. If the mothers had a high concentration of these substances in the blood, the girls started puberty 4 months earlier on average.

For the boys, the data were slightly more difficult to interpret, since some of the perfluoroalkyl substances in the blood were associated with starting puberty 1 month earlier and others were associated with starting 4 months later. Nevertheless, both girls and boys differed significantly in the age at which puberty started according to the concentrations of these substances. And all the various perfluoroalkyl substances – new and old – seemed to affect when puberty started.

"We had hoped that the second generation of these substances had less harmful effects than the old ones, but unfortunately this does not seem to be the case. One might hope that the rules for introducing chemical compounds into general use would be subject to the same strict requirements as, for example, new medicine," explains another main author, Cecilia Ramlau-Hansen, Professor, Department of Public Health, Aarhus University.

Important for children's health throughout life

The new study suggests that more thorough toxicological studies would be appropriate before new substances are approved for use. The new findings are worrying in any case, since previous studies suggest that earlier puberty is associated with increased risk of overweight, cardiovascular diseases, diabetes and some types of cancer, such as breast cancer.

"However, more research is still needed to explain the precise mechanisms that lead to the changes in the puberty profile. One explanation may be epigenetic effects, since we know that substances in our environment and food can affect the surface of DNA chemically."

The chemical modifications of DNA greatly affect which genes are ultimately expressed and when. Evidence also suggests that the substances to which a fetus is exposed during pregnancy greatly affect the child's health throughout life.

"If the perfluoroalkyl substances actually chemically change our DNA, this could be a plausible explanation for what we have found. However, it is still too early to determine the precise underlying mechanism between the chemical compounds, the expression of our DNA and the physiological changes we observe."

"Exposure to perfluoroalkyl substances during fetal life and puberty development in boys and girls from the Danish National Birth Cohort" has been published in Environmental Health Perspectives. In 2014, the Novo Nordisk Foundation awarded a grant to Cecilia Ramlau-Hansen for the project Birth Outcomes and Genital Malformations in Children of Mothers with Pregnancy-Associated Cancer: A Nordic Epidemiologic Cancer Project.

Researchers find link between male sex hormones and ovarian disease

Polycystic ovary syndrome is the most common disorder among women of reproductive age and often leads to problems in becoming pregnant. Now scientists have found a link between polycystic ovary syndrome, elevated levels of male sex hormones, obesity and effects on the fetus.

By Kristian Sjøgren

Polycystic ovary syndrome (PCOS) is the most common disorder among women of childbearing age and often leads to difficulty in getting pregnant. Between 10% and 15% of all women, and up to 25% of obese women, have PCOS.

Now a new study shows that PCOS not only affects women but can also affect their unborn children.

"The high levels of male sex hormones and obesity in PCOS can affect how the placenta functions among pregnant women and thus also the fetus. Our study is the first to examine how PCOS affects all the proteins in both the placenta and in the fetal liver," explains the researcher behind the study, Elisabet Stener-Victorin, Professor, Karolinska Institutet, Stockholm, Sweden.

Elisabet Stener-Victorin recently published the results in the *International Journal of Obesity*.

Examined changes in all proteins in the uterus and the fetus

The symptoms of PCOS include high levels of male sex hormones, which can lead to unwanted hair growth and acne. Women with PCOS also often have difficulty in getting pregnant because their menstrual cycles are irregular. In addition, obesity is very common among women with PCOS, and they have a greater risk of developing depression and anxiety.

In this new study, Elisabet Stener-Victorin investigated using mice how the mother's obesity and elevated levels of male sex hormones affect the function of all proteins in both the placenta and in the fetal liver.

Protein function in the liver and placenta shows how the fetus is developing and under what conditions.

To examine this, the researchers first fattened up the mice, got them pregnant and then gave them high levels of male sex hormones in the last stage of pregnancy. Just before the mice gave birth, the researchers examined the activity of all proteins in the placenta and in the fetal liver.

The researchers found just under 5000 proteins in the placenta, of which 404 were phosphorylated, meaning they are active. In the fetal liver, the researchers found 5400 proteins, of which 474 were phosphorylated.

"We can compare our main finding, which has not previously been found, with the protein activity in mice that have not been exposed to either male sex hormones or fattening diets," says Elisabet Stener-Victorin.

Changing how proteins are expressed

The researchers further examined the protein activity and how this differed between mothers with PCOS and other mothers. One finding was that ATP-citrate synthase proteins were downregulated in the placenta and that catechol-O-methyltransferase (COMT) proteins were expressed differently than normal.

The fetal liver had elevated levels of phosphorylated COMT if the mothers had been obese.

The researchers previously showed among mice that the offspring of mothers with PCOS have a greater risk of developing anxiety symptoms, and the changes in the activity of COMT may contribute to explaining this.

"We previously showed that the offspring of PCOS mice have an increased risk of developing anxiety, and here we showed changes in the mechanism regulating COMT in both the placenta and in the liver. Changes in the environment in which the offspring develop may explain why anxiety develops," says Elisabet Stener-Victorin.

Children of normal-weight women with PCOS remain at higher risk

Although the study involved mice, Elisabet Stener-Victorin says that the knowledge can be used to obtain more insight into how PCOS affects people.

In the long term, the goal is to counteract PCOS in women to avoid the fetus being put at greater risks of also developing PCOS and becoming obese and of developing depression and anxiety.

"Obesity makes everything worse, so losing weight is still important for women who want to become pregnant. However, our experiment shows that male sex hormones are the dominant factor, so this is where we need to intervene for women with PCOS. This can be done using drugs or by lifestyle changes," says Elisabet Stener-Victorin.

Another aspect of this story is that elevated levels of male sex hormones seem to be most strongly associated with problems in getting pregnant. This means that women with normal weight who have PCOS should also talk to their doctor if they want to become pregnant.

"Just a slight weight loss or exercise can probably reduce circulating male sex hormones among women with PCOS, thus reducing the risk of problems in getting pregnant and the risk of affecting the unborn child," concludes Elisabet Stener-Victorin.

"Mice exposed to maternal androgen excess and diet-induced obesity have altered phosphorylation of catechol-O-methyltransferase in the placenta and fetal liver" has been published in the International Journal of Obesity. In 2016, the Novo Nordisk Foundation awarded a grant to Elisabet Stener-Victorin for the project Maternal Androgen and Obesity: Effects on Placenta and Fetus Function, on Offspring Behavior and Metabolism and on Gut Microbiome Function.

Researchers are discovering how paracetamol damages the liver

An overdose of paracetamol can be deadly. Although paracetamol poisoning causes no symptoms initially, it may have catastrophic effects on the liver. These people often feel fine for up to several days after an overdose, but the paracetamol activates a mechanism that slowly but surely degrades the liver. Researchers now believe they have discovered new knowledge about this mechanism. They hope to eventually be able to counteract the liver damage caused by both paracetamol poisoning and other liver diseases.

By Morten Busch

”We theorized that paracetamol poisoning damages liver cells through two steps. The first step is paracetamol and its waste products causing direct damage, whereas the second step is less well known. ”

Cecilie Brøckner Siggaard

Many people decide to take an overdose of painkillers when life becomes too hard and unmanageable. The pills often contain paracetamol which, when taken in large quantities, immediately poisons the body because it kills liver cells.

“We theorized that paracetamol poisoning damages liver cells through two steps. The first step is paracetamol and its waste products causing direct damage, whereas the second step is less well known. Our study suggests that the direct damage from paracetamol activates macrophages – immune system cells – that potentially increase liver cell damage and that the degree of macrophage activation is crucial in determining the outcome of paracetamol poisoning. If this can be confirmed in larger studies, it opens up new options for treatment and monitoring people with paracetamol poisoning,” says a main author, Cecilie Brøckner Siggaard, Resident Physician, Aarhus University Hospital.

Fortunate circumstances complicated the study

Previous studies have primarily investigated paracetamol poisoning at the later stages after people have already developed liver damage, but the researchers wanted to identify the early signs of what occurs when paracetamol degrades the liver after poisoning.

“For people admitted with paracetamol poisoning who subsequently develop liver cell damage, we can measure elevated values of soluble haemoglobin scavenger receptor (sCD163) and soluble mannose receptor (sMR) shortly after the overdose. These markers show that the macrophages have been activated in the liver. These values decline again among the people who do not develop significant liver cell damage.”

However, otherwise fortunate circumstances complicated the study. Fewer people than before are now experiencing paracetamol poisoning because today it can only be purchased in small packages in Denmark and other countries. However, the researchers measured the markers among 49 people with early mild paracetamol poisoning from Aarhus University Hospital and 30 people from the Royal Infirmary of Edinburgh with severe acute liver damage following paracetamol poisoning.

“Among the people with mild paracetamol poisoning, those who showed signs of liver cell damage had significantly higher levels of sCD163 than those who did not. However, the people with acute liver damage from Edinburgh had the highest values. This indicates that sCD163 can help us to identify, at an earlier stage, the people who later develop severe liver cell damage.”

May be a general mechanism

The researchers still do not understand exactly what mechanism is initiated when paracetamol degrades the liver and activates the immune system. Initially, however, the goal was primarily to determine whether they could use these markers to differentiate between people at high risk and people who appear to avoid severe liver damage.

“These markers will potentially enable us to separate these groups relatively easily so that we can focus our efforts on the people at high risk of developing severe liver damage.”

The study also surprisingly provided the researchers with new knowledge about N-acetylcysteine, an antidote to paracetamol poisoning, which reduced marker levels among everyone with poisoning – those who had liver cell damage and those who did not. This was also subsequently confirmed among healthy controls.

“We know that N-acetylcysteine works by neutralizing paracetamol and its waste products directly, but our results indicate that it also presumably directly affects the macrophages. This is new knowledge and can potentially explain why people with other liver diseases treated with N-acetylcysteine experience positive effects.”

However, more studies are required before concluding further on the underlying mechanism behind how N-acetylcysteine works and the importance of macrophages for liver cell damage after paracetamol poisoning. For now, the researchers are delighted that they have apparently discovered a breakthrough that may also be able to help people other than those with paracetamol poisoning.

“We are currently measuring sCD163 among people with viral hepatitis before and after treatment; the values are elevated and then decline after treatment. Previously, we showed that sCD163 is a marker of the severity of fatty liver disease and declines after treatment. If sCD163 is consistently reliable as a biomarker in liver disease in general, we can use these measurements to monitor liver cell damage from a variety of causes.”

“Macrophage markers soluble CD163 and soluble mannose receptor are associated with liver injury in patients with paracetamol overdose” has been published in the Scandinavian Journal of Gastroenterology. In 2014, the Novo Nordisk Foundation awarded a grant to Henning Grønbæk, Department of Clinical Medicine, Aarhus University for the project Inflammatory Liver Diseases and the Role of Hepatic Macrophages.

Solving the mystery of molar miscarriages

Pregnancy is usually a happy time, but for some couples the joy is short lived if the pregnancy is abnormal and results in a miscarriage, which can occur at different times during pregnancy. Molar pregnancies always end up in miscarriages because all or part of the fetus is transformed into a tumour that forms clusters that resemble grapes. Researchers are now discovering the early-stage mechanisms that can result in these unfortunate pregnancies. This new knowledge will make the causes easier to understand and untangle and can eventually lead to the development of treatment.

By Morten Busch

Molar pregnancies can also lead to choriocarcinoma cancer.

The first 3–5 days are critical for a fetus. In that time, the egg develops from one cell into a small, highly organized mass of cells. Some of the cells develop into an embryo, and others form the cells that will make the embryo implant in the uterus. Sometimes, however, something goes completely wrong. In molar pregnancies, the organization of the cells is almost non-existent, which means that these pregnancies usually end with miscarriages. Why these pregnancies go wrong, however, has been a mystery, but now researchers are discovering the very early key mechanisms.

"A defect in the mother's genes causes the early fetus to develop into a molar pregnancy. We are not certain whether there is one effect or more simultaneous effects, but at least we are now discovering the much earlier molecular effects we have struggled so long to find. This means that we cannot merely find the reasons why these pregnancies go wrong. We can also predict them, and perhaps improve treatment in the long term for the people who are affected," explains Karin Lykke-Hartmann, Associate Professor, Department of Biomedicine, Aarhus University.

The project is a longstanding collaboration between Karin Lykke-Hartmann and MD, professor, ph.d. Lone Sunde, Aarhus University Hospital and Department of Biomedicine, Aarhus University.

Mice do not have molar pregnancies

Molar pregnancies result from the abnormal development of the early fetus. In molar pregnancies, the placenta is filled with fluid that resembles grape-like clusters. A molar pregnancy thus only rarely leads to the birth of a child. So far, however, researchers have had great difficulty understanding what goes wrong in early pregnancy.

"Investigating this type of pregnancy has been very difficult since we cannot conduct experiments on fetuses and because mice do not develop molar pregnancies. To examine this more closely, we decided to study more generally how a specific group of genes influences molar pregnancies and miscarriages. Although mice do not develop molar pregnancies, we have managed to identify some of the same dysfunctional mechanisms in mouse fetuses."

The researchers were surprised to discover that genetic defects in some of the cell receptors that are otherwise normally associated with the immune system may be associated with molar pregnancies. These nucleotide-binding oligomerization domain (NOD)–like receptors (NLRPs) are recognized for their important roles in innate immunity and apoptosis (programmed cell death), which ensures that old or malfunctioning (infected) cells die and are replaced.

"These receptors usually recognize bacteria and fungi, but they also seem to regulate embryonic development at the very early stages of pregnancy. This is probably only one of several important mechanisms, but the evidence suggests that, if something goes wrong at this stage, the fetus develops into a clump of cells that lacks the normal cohesion and interaction."

The grape-like cells in a molar pregnancy are the cells that normally interact with the cells in the uterus to ensure proper implantation, a process that is similar to immune system processes, and the NOD-like receptors being present in embryos therefore makes some sense.

Some of these NOD-like receptors are present in the egg before fertilization. As the egg develops, some molecules from the mother's cells are transferred to the egg, which will help both the egg and the embryo to develop. Following fertilization, the egg completely depends on the mother's contribution of molecules for the first 3–5 days. The activity of these NOD-like receptors originating from the mother therefore depends on whether the moderating genes have defects. Curiously, a small group of these NOD-like receptors is already present in the egg before fertilization. A defect in the mother's genes can make the receptors not work optimally, resulting in miscarriages and sometimes the development of molar pregnancies.

Can lead to cancer

Although the NOD-like receptors appear to play a very important role in the early fetal stages, the research by Karin Lykke-Hartmann and her colleagues indicates that other important factors appear to influence whether pregnancies go wrong. Epigenetic signals appear to be one of these very clear factors.

"The number of methyl groups on the DNA strands in the fetal cells is clearly correlated with the development of the fetus in the early stages. So this means that errors in specific NOD-like receptors can cause changes in the epigenetic imprinting, thus destroying the normal regulation of other genes. Epigenetics – how the DNA is chemically modified in the cells – is very important in determining which genes are expressed and thus how the fetus develops."

Thus, genes are especially important for forming the early-stage fetus so that the correct cells are present to produce both the embryo and the surrounding cells that facilitate implantation in the uterus.

Epigenetic defects are common in cancer. This makes the association between NOD-like receptors, miscarriages and the development of cancer much more relevant to investigate.

Molar pregnancies can also lead to choriocarcinoma cancer. Molar pregnancies are fortunately relatively rare and only occur in about 1 in 1000 pregnancies, but their physical and mental effects are so great that the examinations and explanation are very important for parents experiencing a miscarriage.

"It is surprising how little we still know about the early human development , but learning to understand these molecular mechanisms may partly help us to unravel what exactly goes wrong when things go wrong – not only for molar pregnancies but also for miscarriages generally. In any case, our experiment showed that, when we eliminate specific NOD-like receptors in an early fertilized egg, the embryo stops developing prematurely. Although we cannot induce molar pregnancies in mice, we can still learn much about the process. If we are really fortunate and skilled, we may even be able to screen for these changes and eventually even find a treatment."

"The pivotal roles of the NOD-like receptors with a PYD domain, NLRPs, in oocytes and early embryo development" has been published in Biology of Reproduction. In 2017, the Novo Nordisk Foundation awarded a grant to Karin Lykke-Hartmann for the project How Growth and Related Factors Regulate Follicle Activation in Ovaries.

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