Radboud Honours Programme
Medical Sciences 2018

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6500 HB Nijmegen

E.annelies.ruijs@radboudumc.nl
Requests for copies of this book can be sent to the above address.

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Preface

We are very proud to present to you the work of the eighth group of students of the Radboud Honours Programme Medical Sciences.

The honours programme enables second and third year bachelor students in biomedical sciences, dentistry and medicine to expose themselves to an extra challenge and become actively involved in (bio-)medical research.

During the first year of the honours programme (their second bachelor year) the students improved their English writing and presentation skills, and familiarized themselves with the research carried out at the Radboudumc. The three research institutes Radboud Institute for Molecular Life Sciences, Radboud Institute for Health Sciences, and Donders Institute for Neuroscience provided lectures, clinical visits, lab visits and actual hands-on experiments to introduce the research themes and technology centers.

After this introduction, the students started their own research project. For seven months they prepared themselves, under the guidance of a principal investigator, for a research internship abroad of at least three months at renowned institutes all over the world. These extra activities were all completed next to the regular bachelor programme.

From Tel Aviv to Toronto, Cambridge to Clayton, from Dublin to Dallas, and from Milan to San Francisco our students found a warm welcome in the research group of their choice. Being rather inexperienced at this stage of their careers, they had to quickly acquaint themselves with the various (lab) techniques that were required. But with their motivation and ambition to distinguish themselves and to advance their research field, their hard work and the help of their supervisors, all have made their internship a success. Many of our students will be co-author of a scientific publication.

From their personal statements on their honours experience we learn that this has been a period of enormous personal and professional growth. We hope and are also very confident that they will continue to do research to tackle the many questions that still need to be answered in (bio)medical science.

For this book, the students have adapted their internship reports into an article for a broader public. These articles show the great diversity of research fields to which our students have contributed, and we hope that they will be an inspiration to others.

Prof. Dr. Roland Brock
Programme Director
Radboud Honours Programme Medical Sciences

"At my research lab, I worked with virtual reality, which was not only impressive to see and experience, but also very fun to work with."

Araz Aazamy

(Rotterdam, 1996)
Bachelor of Medicine 2015 -2018

Honours Internship:	Department of Psychiatry, Radboudumc; Sagol Brain Institute, Tel Aviv University, Israel
Supervisors:	Nils Kohn, Prof. Dr. Guillén Fernandez; Noam Goldway, Prof. Dr. Talma Hendler

In my first year, I found myself wanting to dig deeper than our medical curriculum allowed. I saw the Honours Programme as a great opportunity to broaden my knowledge, and the idea of going abroad during my Bachelor degree was also appealing.

I did not expect beforehand that I would enjoy the research side so much. I was lucky that both the projects in the Netherlands and Israel were very exciting. Of course, the internship in Israel was the highlight of the Honours Programme for me. Tel Aviv was a great city to study in, and it was crazy to be so close to the beach with three months of sunshine.

At my research lab, I worked with virtual reality, which was not only impressive to see and experience, but also very fun to work with. The people at my lab were great and made my time there more enjoyable. It was inspiring to work together on a project and see it develop from working on the design of the experiment to actually running it with participants, and then be surprised by all things that still go wrong on the first run.

All in all, I have enjoyed and learned a lot in the past two years. And I'm happy to say that my experience has definitely raised my interest in doing research in the future!

A new method for measuring body ownership implicitly

Araz Aazamy

Although most of us take our self-consciousness for granted, it is actually quite astonishing. Human adults experience a unitary entity; the 'I' or 'a real me' that resides in our body and is subject to thoughts, bodily signals, and the surrounding world. As one might imagine, studying self-consciousness is challenging. Therefore, an alternative, but powerful method to gain more insight into self-consciousness is studying brain mechanisms that are involved in bodily self-consciousness.

Bodily self-consciousness

Bodily self-consciousness consists of three aspects: self-identification (feeling ownership over a body part or the entire body), self-location (where 'I' believe to be in space) and a first-person perspective. In healthy individuals, our bodily self-consciousness is usually limited to our own body. As the latter suggests, there are exceptions. Previous research has shown that several aspects of bodily self-consciousness can be altered. A well-known example of this is the rubber hand illusion (RHI). In this experiment, the participants' hand is hidden from sight, and a rubber hand is placed in front of them on a table. Subsequently, both the real and rubber hand are stroked simultaneously and synchronously by a brush. The experiment ends with a hammer hitting the rubber hand, which usually makes the participant pull the real hand away fearfully.
The reason people fear that the hammer will hit their hand is that the simultaneous and synchronous stroking of the rubber hand and the real hand induces illusionary ownership (self-identification) for the rubber hand. After a short period of stroking, participants will start to feel the touch of the brush on their actual hand at the location where they see the touch is applied. Also, the location where the real hand is perceived to be (self-location) tends to shift towards the rubber hand; a phenomenon called proprioceptive drift. This experiment demonstrates that our bodily self-consciousness is not limited to our own body. That is because the brain uses sensory inputs and bodily signals to determine aspects such as self-identification and self-location.

Virtual reality

Luckily, our brain is quite accurate most of the time due to the continuous presence and renewing of bodily signals and our senses. However, this also makes studying bodily self-consciousness challenging. In recent years, improvements in virtual reality have enabled experimentation in virtual environments in which multisensory information concerning both the position and presentation of the body is ambiguous. These new developments have opened up the door to investigate the different aspects of bodily self-consciousness further in complex and dynamic virtual environments.

However, the full potential of these technologies is hard to exploit due to the fact that they have superceded the available methods for measuring body ownership. Currently, this is measured using questionnaires and proprioceptive drift. Although these are valid methods, an ongoing experiment must be stopped to assess ownership rather than measuring this implicitly. Therefore, the project I joined aimed to find a measure for body ownership that is implicit and direct. A potential candidate for this is specific ERPs (event-related potentials) which can be measured using EEG (electroencephalography). Previous research has shown that violations of bodily movements (movements that are not caused by participants themselves) of a virtual body can elicit such a specific ERP. In this research, participants were embodied in a virtual avatar and performed a task in which they had to grab an object in a virtual environment. Real movement errors (e.g., to miss the object) and correct movement responses (i.e., to grab the object) by participants elicited a similar ERP as the one observed during violations of bodily movements. However, the amplitude of the ERP was significantly higher during violations of bodily movements compared to real errors and correct responses. This kind of ERP, which can be triggered by violating bodily movements, might be linked to body ownership. The research I was involved in aimed to find such ERPs that could act as a fingerprint for ownership. For this, we designed an experiment with five different conditions which test and control for body ownership. However, due to the limited time scope of my internship, my goal was to examine the behavioral data of these five conditions to compare for ownership.

Body ownership induction and control conditions

Sixteen individuals, mostly students from Tel Aviv University, participated in our study. The participants were hooked up to the necessary equipment, including four movement trackers to represent the movement of the hands in the virtual environment, an HTC VIVE (a virtual reality headset), and the EEG equipment. In the virtual environment, the participants were presented with an empty room and a table in front of them as if they were sitting on a chair. This scene was the same for every condition. Also, their upper limbs were represented. Important to note is that the arms were elongated and did not match the exact location in reality. In total, the experiment consisted of five conditions. The order of the conditions was randomly selected for every participant.

The first body ownership induction condition was a virtual variant of the RHI described above. The condition consisted of eight stroking tasks, during which one of the hands of the participant was stroked with a cotton swab, and eight movement violation tasks in which participants were instructed to put their hands on the table and saw their hands jump randomly. During the latter task, ERPs could be measured in response to the jumps. The two different tasks alternated during the condition. The control condition was identical to the condition described above, except the stroking was, unlike in the RHI, asynchronous. This action is known to break or reduce the illusion.

The second body ownership induction condition was identical to the first one, except the stroking task was replaced by a task in which participants were instructed to slide the palm of one of their hands over a two-directional arrow sign displayed on the table. The first control condition was identical to the induction condition, except the upper limbs were replaced by cursors. Research has shown that when an object is used instead of a body (part), the illusion does not work. The last control condition was also identical

to the second induction condition, except the upper limbs were represented in a true mirrored manner at the opposite side of the table. The reason we included this condition is to control for ERPs that can occur when we see others making an error.

Body ownership questionnaire and proprioceptive drift

Between the conditions, participants answered a 16-statement questionnaire, which includes four questions that test for ownership and four questions that control for ownership. Participants could fill in a score for each statement, ranging from -3 (strongly disagree) to +3 (strongly agree). We used a paired t-test to test for significance between the ownership scores of the conditions. Furthermore, we measured whether any proprioceptive drift occurred. The proprioceptive drift was measured at the beginning and end of every condition; participants performed a task in which their hands were not represented, and were asked to move a dot in the middle of their screen to the location where they believed their hands were. We compared the mean proprioceptive drift of the ownership induction conditions to their corresponding controls to test for significance.

Results and conclusions

We expected to find significantly greater mean proprioceptive drift and ownership statement scores for the two body ownership induction conditions compared to their corresponding control conditions. The results we found partly confirm our expectations. The experiment shows that the mean proprioceptive drift in one of the ownership induction conditions (the virtual RHI condition) was significantly higher compared to the control condition. However, the difference between the mean ownership questionnaire scores of both conditions was not quite significant. This difference might become significant if more participants are included, supporting the use of this ownership induction condition as a reliable method to induce body ownership.

As for the remaining conditions, we saw an opposite effect. The proprioceptive drift between the conditions was not significant, but the mean questionnaire ownership scores between the test and control conditions were significant. Although the non-significant results are not in line with our expectations, these results do not necessarily contradict our expectations. However, with the results at hand, it is difficult to determine whether the ownership induction conditions and their corresponding control conditions have their desired effect, that is to induce and control for body ownership, respectively. For the first induction condition and its control condition, the results are promising. However, it is difficult to determine whether the ERPs measured in the conditions have any value at this point. With more participants, reliable results may be obtained, which will bring us a step closer in understanding bodily self-consciousness.

Special thanks to
Prof. Dr. Guillén Fernandez and Nils Kohn for being great and supportive supervisors, and to Prof. Dr. Talma Hendler for hosting me in Israel and Noam Goldway for letting me join such an interesting project.

Figure 1. Experimental setup.

"By working directly with patients, I got to know a lot of stories and struggles. This human element gave a sense of purpose to the research I was doing."

Kevin van den Berg

(Amersfoort, 1996)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Neurology, Radboudumc/Donders Institute; Human Motor Control Section, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland, USA
Supervisors:	Dr. Rick Helmich; Dietrich Haubenberger, MD

To be honest, there were times when I wasn't too sure about my decision to participate in the Honours Programme. This was especially true during the last few weeks before my departure to Washington DC. I had an exam coming up, but I still needed my visa, a room, and a plane ticket. However, when I stood at the Lincoln Memorial and saw the beautiful view over the National Mall with the Washington Monument standing in line with the U.S. Capitol I knew I had made the right choice.

My experience at the Human Motor Control Section at the National Institutes of Health (NIH) was amazing. I had the opportunity to work with medical doctors, neuroscientists, and students from all over the world. My job at the NIH was to finish a study my supervisor had started a few years back. In this study we wanted to know what brain regions are generating postural tremor in Parkinson's disease. The most fascinating part of this project was that I was working with human subjects. People flew from all over the U.S. to the NIH to participate in studies. By working directly with patients, I got to know a lot of stories and struggles. This human element gave a sense of purpose to the research I was doing.

I feel very fortunate in having participated in the Honours Programme. It provided me not only with essential knowledge on medical research but also with new friendships overseas and experiences that I will never forget. In my view, this is what the programme is about.

What drives postural tremor in Parkinson's disease?

Kevin van den Berg

In 1817, James Parkinson was the first to publish a detailed description of Parkinson's disease (PD) in An Essay on the Shaking Palsy. PD is a common neurodegenerative disorder characterized by slowness of movement, stiffness, postural instability and shaking of limbs (tremor). With tremor being one of the striking features, Parkinson included 'shaking' in the title of his article. Even so, after 200 years the disease mechanisms of tremor remain poorly understood.

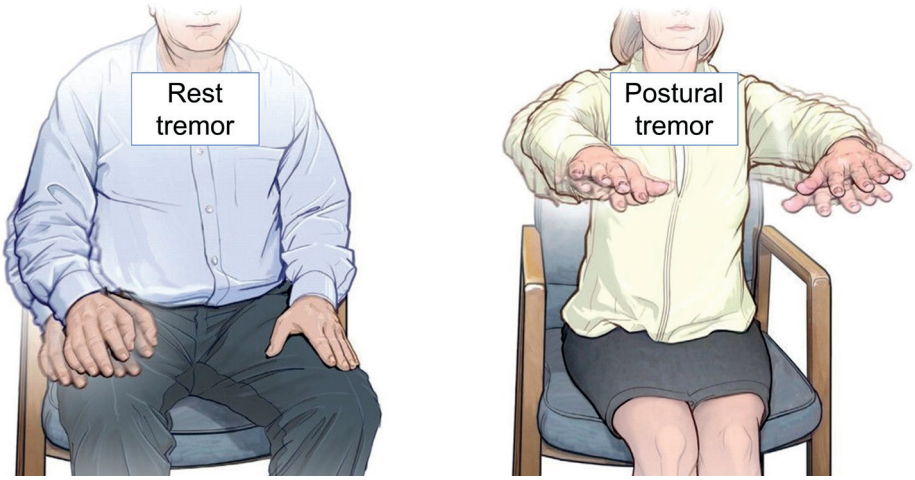

Figure 1. Rest and Postural Tremor
Adapted from: "Essential tremor: Choosing the right management plan for your patient" (2011)

Postural tremor in Parkinson's disease

Tremor in PD often occurs in the hands during rest. However, tremor can also occur when patients hold their arms against gravity. This is called postural tremor (see figure 1). There are two types of postural tremor in PD, called 're-emergent' and 'pure postural' tremor. In re-emergent tremor we see that tremor disappears during movement. You can see this during the transition from rest to posturing, such as stretching out your arms. You will see that the rest tremor suddenly stops when patients start stretching out their arms. When they hold their arms in front of them, tremor reappears after a few seconds. The frequency of this tremor is the same as rest tremor and like rest tremor dopamine seems to reduce its amplitude. It is believed that this type of postural tremor is a rest tremor that re-emerges during posturing, hence its name. In pure postural tremor we don't see the

tremor disappearing during movement. Tremor appears directly after assuming a posture. The frequency of this postural tremor is higher than rest tremor and there is no effect of dopamine. This raises a few questions. First, do rest and re-emergent tremor arise from the same brain regions? Second, does re-emergent tremor arise from a different brain region than pure postural tremor?

The origin of tremor

Unfortunately, it has remained a difficult task to find the brain regions that cause tremor. However, modern technologies are providing potential solutions. Functional MRI (fMRI) can be used to locate brain regions that are activated during tasks or movements. Electromyography (EMG) can be used to measure muscle activity. Using these techniques, a tremor network consisting of various brain regions has been proposed. Within this network there are two brain regions that may be involved in the generation of tremor. These brain regions are the motor cortex (M1) and the cerebellum. M1 is involved in the execution of voluntary movements. The cerebellum is involved in coordination and fine adjustments of movements.

One limitation of fMRI is that it cannot accurately show whether brain regions activate the tremor or whether they are activated due to the tremor. Transcranial magnetic stimulation (TMS) can be used to assess this causality. TMS is basically an electromagnet and by placing this over a brain region and quickly turning it on, a strong magnetic field is suddenly produced. This magnetic field passes through the skull and can electrically stimulate the underlying brain region (see figure 2). If a magnetic pulse over a brain region changes muscle activity in the tremulous muscles, that brain region is generating or transmitting tremor. This change in muscle activity can be calculated using the tremor reset index (TRI), which is a value that goes from 0 to 1. It shows how much a tremor can be changed or 'reset' when TMS is applied over a certain brain region. Zero means the brain region is not generating or transmitting tremor and 1 means the brain region is fully generating or transmitting tremor.

Tremor study at NIH

At the National Institutes of Health (NIH) we started a study to investigate the role of M1 and the cerebellum in Parkinson's postural tremor. We used EMG to record tremulous muscle activity whilst applying TMS over M1 and the cerebellum (see figure 2). We used the TRI to assess whether these brain regions are involved in the generation or transmission of tremor. First, we distinguished between re-emergent and pure postural tremor. We looked whether tremor disappeared during the transition from rest to posturing. For the purposes of this study, we defined posturing as wrist extension with the forearms supported on the armrests of a chair. We also compared tremor frequencies during rest and posture. We then assessed M1 and the cerebellum for both rest and postural tremor. A strength of our study was that we timed our TMS pulses relative to the onset of postural tremor. We gave a TMS pulse 2 and 12 seconds after tremor reappeared during posturing. This way we could also investigate when brain regions become active relative to the onset of tremor.

Distinguishing between re-emergent and pure postural tremor

We included 11 subjects in this study. Of these 11 subjects, 10 had re-emergent tremor and one had pure postural tremor. In the re-emergent group, the tremor clearly disappeared during the transition from rest to posture. Having assumed a posture, the tremor reappeared after about 5 seconds. Tremor frequencies during rest and posture did not differ. In the pure postural patient, tremor did not disappear during the transition from rest to posture; it appeared immediately after posturing. In addition, tremor frequencies were higher during posture compared to rest.

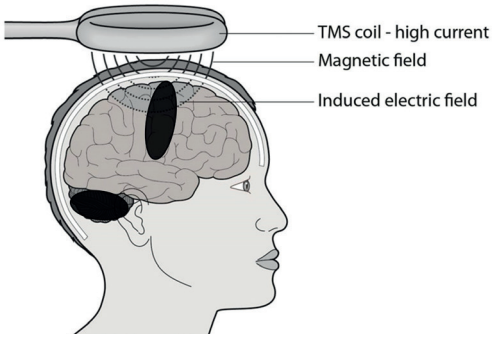

Figure 2. Transcranial Magnetic Stimulation. TMS was applied over the motor cortex (1) and the cerebellum Adapted from: "TMS may Help Improve Memory" (2017)

Resetting tremor

Clear differences were found between M1 and the cerebellum when it came to resetting tremor. Figure 3 shows the TRI's in the re-emergent group. In this group, we were able to reset rest and postural tremor by applying TMS over M1. This suggests that M1 plays a similar role in generating or transmitting rest and re-emergent tremor. When we compare the TRI's of early and late stimulation of M1, we see no significant differences. This means that M1 generates or transmits tremor right from the start. Its role in generating or transmitting tremor does not differ relative to the onset of the tremor.

We weren't able to reset tremor by applying TMS over the cerebellum. This suggests that the cerebellum is not involved in generating or transmitting rest or re-emergent tremor. However, when we compare early and late cerebellar stimulation, we see that late stimulation gives a higher TRI value. This is an interesting finding. It means that the cerebellum plays a more important role late after tremor onset. However, the TRI value is not high enough to draw definite conclusions. More data is needed to support this idea.

Unfortunately, we cannot compare the TRI's from re-emergent tremor to pure postural tremor as we have only had one pure postural patient. Preliminary data from the pure postural patient indicate a similar involvement of M1, but of interest are higher TRI values for the cerebellum. This suggests a higher involvement of the cerebellum in pure postural tremor. Currently, researchers at the NIH are continuing this study in order to further elucidate the role of M1 and the cerebellum in postural tremor in PD.

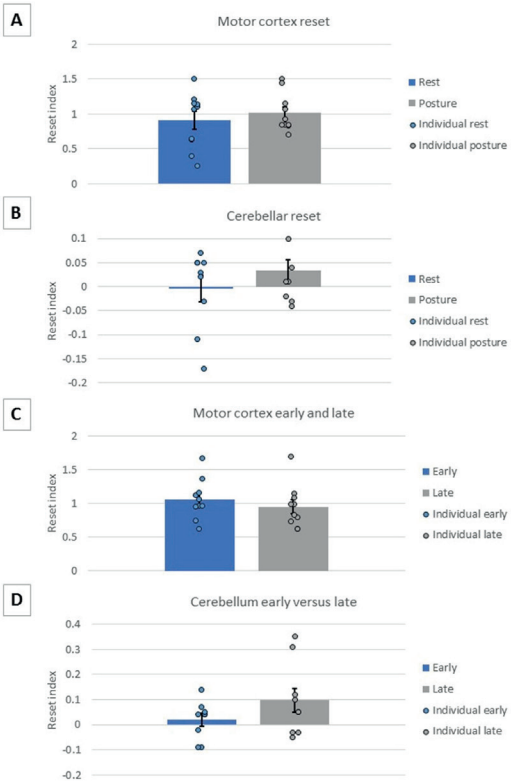

Figure 3. Average tremor reset indexes in re-emergent group for rest and posturing including individual data points and standard error of the mean.

- *Figure A shows the reset indices for M1 stimulation during rest and posturing.*
- *Figure B shows the reset indices for M1 stimulation during early and late stimulation.*
- *Figure C shows the reset indices for cerebellar stimulation during rest and posturing.*
- *Figure D shows the reset indices for cerebellar stimulation during early and late stimulation.*

The future of tremor

Tremor in Parkinson's Disease has proven to be a difficult symptom to unravel. It has been more than 200 years since the first publication on PD and we are still unsure what brain regions are causing tremors. However, every year we are answering more questions and coming closer to understanding the intricate brain circuits involved in tremor. We hope this research may one day provide better targets for future treatment.

Acknowledgements

I would like to thank my supervisor Dr. Rick Helmich for giving me the opportunity to work on this fascinating research topic. His mentorship has provided me with all the knowledge and skills I needed for my internship abroad. In addition, I would like to thank Dr. Freek Nieuwhof for everything he taught me about tremor and coding. A big thanks to Dietrich Haubenberger, M.D., for setting up the study at the NIH. Special thanks to Mark Hallett, M.D., for giving me the chance to work in his lab. Finally, I would like to thank Thomas Osterholt for his help on the tremor protocol.

"Living abroad has taught me many things about myself... For instance, I have learned that I am very happy to be Dutch/ European and how to survive an overkill of sugar."

Marjolein D. van Borselen

(Vleuten-De Meern, 1997)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Pharmacology & Toxicology, Radboudumc; Department of Bioengineering, University of California, San Francisco
Supervisor:	Prof. S.N. de Wildt; Prof. K.M. Giacomini

The Radboud Honours Program Medical Sciences has been a great opportunity to develop both my skills and diverse interests in the medical field. I enjoyed the short intern rotations as which served as an introduction to the Radboudumc Research Institutes and their Themes. These allowed me to discover a modest part of the broad research field of the Radboudumc during my second year of my bachelor's degree.

I was very fortunate to start my individual research project under the supervision of Prof. Saskia de Wildt. She is a very pleasant and intellectual professor and kept me motivated during the inevitable setbacks of the experiments. The highlight of the Honours Program was my internship in San Francisco. I have learned many new techniques, and Prof. Kathy Giacomini allowed me to work on both projects of my interest, for which I am very thankful. Thus, I have worked with two different and very intelligent supervisors, who showed me various aspects of the scientific world. The results of all three projects are promising and I hope they will be published and well-received by other researchers.

Finally, living abroad has taught me many things about myself, as well as living with people of different cultural backgrounds. For instance, I have learned that I am very happy to be Dutch/European and how to survive an overkill of sugar. Nevertheless, the USA is a country with many opportunities and tremendous natural beauty.

I would like to thank the board of the Honours Program Medical Sciences, Saskia, Kathy, and my colleagues from the labs in Nijmegen and San Francisco for this wonderful time and great experience!

Deorphanizing membrane transporters of the kidney

Marjolein van Borselen

How do drugs arrive at the right place in our body? And after the drugs have done their job, how do they leave our body? This is all handled by membrane transporters. For instance, the organic cation transporter 1 (OCT1) is involved in the disposition of morphine and tramadol. Recently, new transporters have been discovered with unknown function. They are called orphan transporters, because we don't know anything about them, just like an orphan knocking on the door of orphanage. Researchers are trying to deorphanize these transporters.

Orphan transporters in the kidney

Some of these orphan transporters might be located in the kidney. The kidney is the filter of our body. In the glomerulus, a functional part of the kidney, the waste and toxic compounds in our blood are filtered and urine is formed. Not all waste is filtered and not all the filtered compounds in the urine are waste. The body has transporters to facilitate movement of these compounds from the blood to the urine and the other way around to create the final urine that we pee. These transporters are coded by our genes, which means that the design of the transporter is encoded in our DNA (fig. 1). The mRNA represents a set of instructions, and is made of our DNA. With these instructions, a protein will be formed by stringing beads-like amino acids, and folding this chain. In this way, transporters are formed with their own specific amino acid combinations. The body has a variety of transporters with different functions and compound specificity. The research field of pharmacogenetics studies this specificity by determining the location, substrates and drug interaction of transporters, shortly the function of a transporter. Lately, a part of the DNA is discovered with genes within the SLC22 transporter family that encodes 9 orphan transporters, in explanation no known compounds nor the localization for these transporters are yet identified.

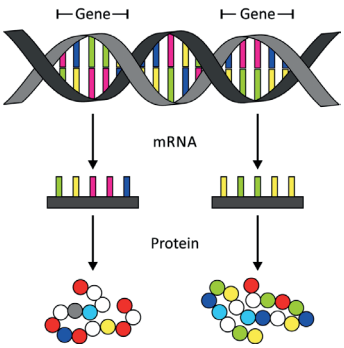

Figure 1. Gene expression begins with DNA and results in proteins like transporters

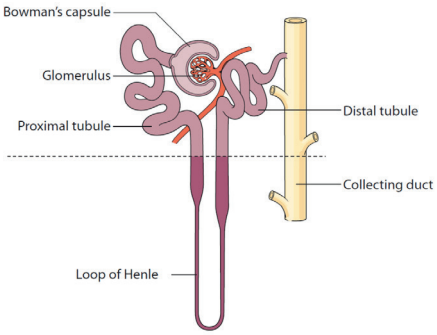

Figure 2. Nephron structure

SLC22 family

Several members in the SLC22 family are known to play an important role in the kidney to secrete or reabsorb compounds including drugs. Members in this family demonstrate a remarkably wide substrate specificity, like the organic anion transporters (OAT) and the organic cation transporters (OCT). They are polyspecific, which means that they transport negative, positive and bipolar ions. They have a critical role in transporting solutes involved in energy production in our cells such as carnitine and thiamine. Furthermore, mutations in SLC22 family members cause rare and harmful diseases like systemic primary carnitine deficiency and renal hypouricemia. Therefore, it is important to determine the function of the 9 orphan members within the SLC22 family, because they might have a critical role in health and diseases. The SLC22A24 is one of these orphans and results of a recent study give more insight in the function of this transporter.

SLC22A24; where are you?

In the process of deorphanizing a transporter, finding the location of the transporter in the human body can give a first clue. The SLC22A24 transporter is mostly expressed in the kidney. The kidney consists of physiologic units, called nephrons (fig. 2). Most drug transporters are in the proximal tubules of the kidney. However, data show that SLC22A24 is primarily located in the collecting duct of the kidney. The collecting duct is the place where the last compounds are transported in or out the urine, before the urine goes to our bladder and eventually into the loo. Besides the divergent location of this transporter, the amount of the transporter found in our body is very low compared to others. This could be explained in different ways. One theory is that as it is located only in a very small part of the body, not many transporters are needed. Another explanation is that perhaps the transporter is gradually disappearing out our body. In explanation, many people have got a variant of the SLC22A24 gene that isn't making a working transporter as described above (fig. 1). This mutation seems to be dominant and so we are losing SLC22A24, like less and less people are being born with red hair. Besides the unknown location of the transporter in our body, we don't know on which of the cell membranes, the outer layer of the cell, the transporter is located. Transporters can be on the membrane at the blood side or the urine side of the cell. Unfortunately, information is missing to figure out the exact location.

Other species with SLC22A24

In genetic research, human genes are often compared with genes of other species, like chimps and rats. This can give insights into the function and evolutionary changes in the genetics of the transporter. Human genes are often similar to the mouse and rat variant. Interestingly, the gene of our transporter isn't like the one in mouse or rat. The SLC22A24 gene is most similar to the gene in chimpanzees, followed by orangutans and rabbits. This information can help researchers understand the function of the orphan transporter. On the one hand, SLC22A24 must transport a compound that is necessary in humans, chimps, orangutans and rabbits. On the other hand, this compound isn't in mouse or rat. A study has shown that steroid glucuronides, a kind of hormone, are only found in monkeys, humans, rabbits and guinea pigs and to our knowledge not in other species, like mice or rats. Thus, the next step is to find out if SLC22A24 transports these steroid glucuronides.

Finding substrates of SLC22A24

For determination of the substrate specificity of SLC22A24, radioactive uptake assays have been performed for a variety of compunds. First, we cultured cells with only the SLC22A24 transporter on their membrane. Then, a fluid with a radioactive labelled compound was added to the cells. If the transporter is specific for the compound in the fluid, the compound will be able to move into the cells. To see if this is the case, the fluid is removed from the cells after a certain time and then the cells are washed. Finally, the amount of radioactive compound that is in the cells is measured. This tells us how specific the compound is for the transporter, in other words, how well the substrate fits through the gap of the transporter. For instance, if the compound was rectangular it wouldn't fit in a transporter with a round gap. Multiple compounds were tested in this way. It turned out that the SLC22A24 transporter particularly likes negatively charged substances. More specifically, it seems to transport steroid conjugates, among which are the steroid glucuronides, and bile acids.

Testing the substrates of a transporter is expensive, because radiolabelled compounds are high-priced. Therefore, a method is created to test non-radiolabelled compounds. In this case, a fluid of a known substrate of the transporter which is more affordable is used as radiolabelled compound and mixed with the non-radiolabelled substrate that needs to be tested. The transporter on the cells will transport the radiolabelled compound or the other substrate. In the end, the amount of radioactive compound in the cells will be compared with the amount of radioactivity in the cells of a separate experiment with no extra substrate in the fluid. This is called competitive inhibition, because both substrates compete to get in the cell. The most specific substrate of the transporter will be transported in larger amounts. While this address the problem of cost, testing compounds as inhibitors isn't equivalent to testing compounds directly as substrates in an uptake assay.

Inhibition experiments have been performed for the SLC22A24 transporter (fig. 3). Different compounds are selected to determine the substrate specificity of the transporter. The selected compounds are negatively charged and have a similar chemical structure. Two drugs, lesinurad and rosiglitazone have been tested as inhibitors of SLC22A24, but based on calculations of the maximal concentration of the drug in our body and the inhibition results, lesinurad and rosiglitazone are unlikely to cause a mentionable interaction with the transporter. The steroid glucuronides seem to give a weak inhibition, because the line in the graph is more at the right in the figure compared to strong inhibitors as progesterone (fig. 3). On the other hand, the tested bile acids, chenodeoxycholic acid and ursodeoxycholic acid, show similar potency in inhibiting the uptake of the radiolabelled substrate. Furthermore, the retinoic acids are vitamin A metabolites and have the weakest inhibition compared to the other selected compounds. Unfortunately, limited conclusions can be drawn from the results of these inhibition experiments. Nevertheless, we can conclude that the compounds are substrates of the transporter, because some inhibition has occurred. The next step is to test the selected substrates as radiolabelled compound and not as inhibitor to give more insight in the potency of the transporter for these substrates.

Is SLC22A24 deorphanized?

The orphan SLC22A24 is located primarily in the collecting duct of the kidney, is similar to the transporter in monkeys and rabbits, and transports steroid conjugates and bile acids. Different compounds have been tested as competitive inhibitors and have given more insight in the function of the mysterious SLC22A24. Competitive inhibition results are less informative than direct substrate testing. Nevertheless, knowing that SLC22A24 transports steroid conjugates and bile acids is one step further in unravelling the function of this transporter. Moreover, the results of the steroid glucuronides as substrates of SLC22A24 with the similarity of the transporter in different species is a breakthrough. Thus, I think we can say that SLC22A24 is now deorphanized!

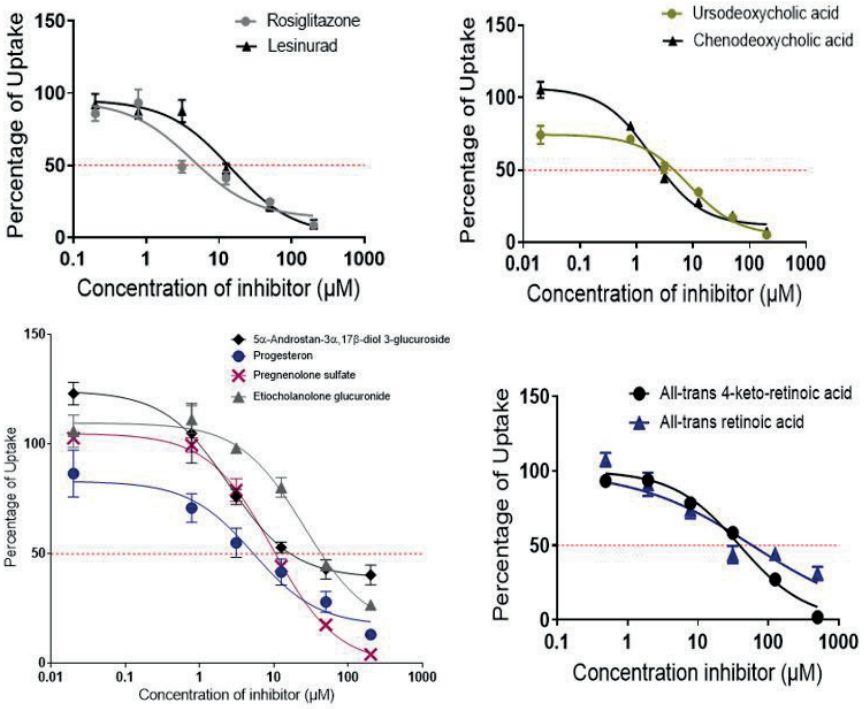

Figure 3. Selectivity of inhibitors of SLC22A24 in stable overexpressed HEK293 cells. Selected drugs bile acids, steroids and retinoic acid are plotted separately in 4 figures.

Acknowledgements
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"I really enjoyed my project, so this internship has confirmed that I am on the right track."

Willem Bosman

(Nijmegen, 1997)
Bachelor of Biomedical Sciences
2015-2018

Honours Internship:	Department of Physiology, Radboudumc; Centre for Mineral Metabolism and Clinical Research, UT Southwestern, Dallas, TX, USA
Supervisors:	Jeroen de Baaij, PhD; Silvia Ferrè, PhD, Orson W. Moe, MD

When I first heard about the Radboud Honours Programme Medical Sciences, I immediately knew it could be a great opportunity. I believed that the programme would be a nice introduction to biomedical research and that it would help with improving my scientific skills and getting in contact with knowledgeable people. Luckily, it has lived up to these expectations.

I started my honours internship at the department of physiology in Nijmegen. It was a valuable addition to the regular bachelor programme and I am especially happy with how many techniques I learned during this time. Because of this, I was able to start my research abroad without much extra training, leading to a more efficient internship. On top of this, Jeroen de Baaij was a very supportive and inspiring supervisor who not only helped me with the lab work, but also with feedback and application forms that were required for my studies.

In March 2018, I finally travelled to Dallas, Texas, where I was immediately greeted enthusiastically by my supervisor Silvia Ferrè. She and Dr. Moe made me feel very welcome in the city and at their department. Silvia provided great guidance throughout my project, but also allowed me to do a lot of experiments independently, which helped me to grow as a researcher. I really enjoyed my project, so this internship has confirmed that I am on the right track. Also, experiencing the differences between the Netherlands and the USA, in research and in general, was very interesting.

Because of the Honours Programme, I have grown as a researcher, I have a clearer view of my interests, and I have had the opportunity to work abroad in a very early stage of my career. Therefore, I would not hesitate to recommend the programme to any (bio)medical or dentistry student.

Magnesium as a regulator of the crucial protein α-klotho

Willem Bosman

In Greek mythology, three sisters, known as the Three Fates, were responsible for the thread of human life. Clotho, one of these sisters, bore the burden of spinning the thread, which gave her great control over the lives of others. She determined who was born and how the lifespan of people would unfold. So if a research group in the modern time of fast-growing biomedical knowledge decides to name a newly discovered protein after this incarnation of destiny, it better have some seriously life-changing effects. This was indeed the case in 1997, the year of the discovery of the protein klotho, nowadays called α-klotho. The reason that this protein was named after the Fate Clotho is clear, as mice that lacked the protein were found to have a drastically shortened lifespan. This indicates that α-klotho has anti-aging effects and a significant impact on the thread of life. The researchers were right: Clotho seems to have found an equal, in the form of a protein.

The importance of α-klotho in kidney disease

α-klotho is mainly present in the kidney, but can also be found in the bloodstream, which means it has effects throughout the entire body. One of the most striking effects of α-klotho, besides its impact on aging, is in chronic kidney disease (CKD). CKD is characterized by a decreasing kidney function which, if left unchecked, will eventually lead to complete loss of function of the organs that are responsible for filtering toxic compounds out of our body. At this point, only three options remain: dialysis, kidney transplantation, or death. α-klotho seems to be a major player in this severe disease. Normally, levels of α-klotho in our bodies stay at a constant value, but in patients with CKD, α-klotho levels drop below this constant. The more the level drops, the more severe the disease and the higher the risk of developing complications like stiffness of the blood vessels. Therefore, it is vital to study how α-klotho is regulated and what factors could influence levels of α-klotho in the body. Not only would this help improving our understanding of CKD disease progression, but it would also lead to possible new treatment options that will improve healthcare for CKD patients.

Potential role of magnesium

One candidate that could regulate α-klotho is magnesium. Magnesium is more abundant in our body than many people may think. It is present in every cell and has a large variety of functions. Similar to α-klotho, magnesium is associated with protection against CKD. Higher levels of blood magnesium lead to a decreased risk for developing CKD and its complications. Various explanations for this exist, but how magnesium works exactly is not fully understood. So what if the protective effects in CKD patients can be explained by magnesium influencing α-klotho levels? That is the question we aimed to answer in this project.

Mouse model experiments

In this study, we used a mouse model to simulate what happens in the human body. In order to study the effect of magnesium on α-klotho levels, we fed 10 mice a diet with normal magnesium content (NMg) and the remaining 10 a diet with low content (LMg). All the mice received their diets for two weeks, after which they were sacrificed and organs, including the kidneys, were collected. Like any other protein, the genetic information for α-klotho is present in the DNA, the genetic code. Therefore, we isolated the part of DNA that is specific for α-klotho and measured how abundant this part was in both the NMg and the LMg group. We also measured the levels of α-klotho protein in the kidneys of all mice. This allowed us to study whether the amount of magnesium intake influenced the abundance of α-klotho DNA and protein levels.

Main results

First of all, it was important to ensure that the diets were effective and that the LMg diet truly led to magnesium deficiency in the mice. This was indeed the case. In both blood and urine, magnesium levels were lower in the LMg mice compared to the controls who received the normal diet. After measuring the α-klotho DNA and protein levels in both groups, we found an interesting and crucial difference: α-klotho levels were lower in the mice that were fed the low magnesium diet. The difference was moderate, but consistent. This indicates that magnesium is required for a proper α-klotho balance. The lower the magnesium content in the body, the lower the α-klotho levels. This main finding and the experiment that led to it are summarized once more in Figure 1 below.

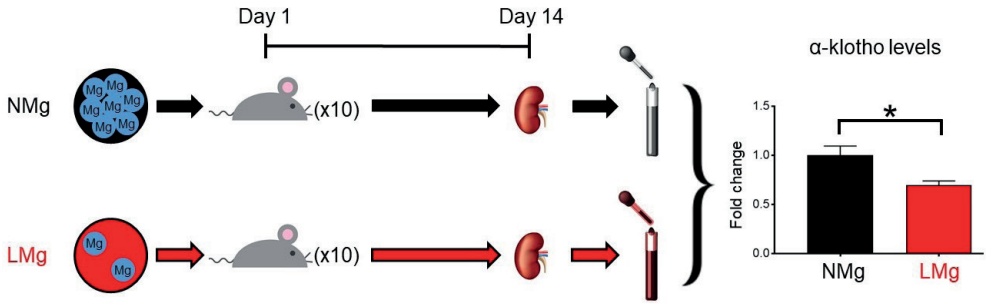


Figure 1. Summary of experiment and main finding
Mice were fed either a normal magnesium (NMg) or a low magnesium (LMg) diet. After two weeks, we sacrificed the mice and collected the kidneys. Further tests showed that α-klotho levels in the kidney of mice fed the LMg diet were decreased. The asterisk indicates that this finding was statistically significant.

Implications for patients

So what do our findings mean for the future of healthcare? First off all, when conducting experiments in mice and animals in general, it is important to remember that the outcomes will not always precisely translate to humans. Biological processes can differ substantially between species, so whether a similar role for magnesium in the regulation of α-klotho exists in humans needs to be confirmed. However, it is known from previous experiments in mouse models that α-klotho behaves similarly in mice and humans. Therefore, our findings do highlight the importance of sustaining sufficient levels of magnesium, especially in patients with CKD, for whom higher levels of α-klotho are

particularly beneficial. So Magnesium could be considered as an additional treatment option for these patients. Clinical studies should be performed to test this.

Implications for future research

In our study we have shown that magnesium regulates α-klotho in the kidneys of mice. What remains unknown is how magnesium does this. Does it influence the part of the DNA that houses the information for α-klotho? Does it bind to the α-klotho protein itself? Or is it something else? As part of the project, we tried to study a possible mechanism for the interaction between magnesium and α-klotho in cell lines, but we could not draw definite conclusions. The main aim for future research should be to understand this mechanism. In this way, we will enhance our understanding of the regulation of α-klotho and we could identify other factors that help magnesium in regulating α-klotho. These new factors could then be other potential treatment targets for CKD patients. In conclusion, our study was the first step in identifying a new mechanism for α-klotho regulation, which contributes to better understanding of CKD disease progression and to finding possible new treatment options for CKD patients.

Acknowledgements
I would like to give special thanks to Silvia Ferrè for her supervision and trust, but also for helping me with finding an accommodation and getting to know the city and campus. I also want to thank Dr. Moe for allowing me to work on this project at his department and sharing a lot of his knowledge. Furthermore, I am very grateful to Jeroen de Baaij for preparing me and teaching me many of the required techniques and skills.

Demi van Dalen

(Nijmegen, 1996)
Bachelor of Medicine 2015 – 2018

Honours internship:	Department of Surgery, Radboudumc; Department of Surgery, University of Chicago, USA
Supervisors:	Prof. Dr. Harry van Goor; Dr. John Alverdy, Olga Zaborina

After a lot of hesitation about applying for the honours programme, I submitted my application letter an hour before the deadline. I have always been interested in research, but I was not sure if it was really for me. Could I combine it with my regular programme or would it be too challenging? Looking back, submitting my letter was one of the best choices I have ever made. The several small internships during the first year taught me all kinds of laboratory skills and each time made me more enthusiastic about starting my own project abroad. At first, I was mainly interested in doing clinical research, but throughout the programme I learned that my interest lies in fundamental science.

In April 2018, it was finally time for my internship abroad. Since it was my first trip to the United States, I had no idea what to expect from a big city like Chicago. But as soon as I arrived and saw the impressive skyscrapers, I fell in love with the city.

In Dr. Alverdy's lab, I got the opportunity to work on several projects, such as those on pancreatitis and sepsis, and learned a lot of skills that will come in handy in the future. The supervision I received during my internship was great; my colleagues were so friendly and Dr. Alverdy is one of the most inspiring and enthusiastic people I have ever met. My internship had its up-and downsides, but I learned not to throw in the towel, because there is always somehow a way forward.

I had a great time and it was over way too soon. I enjoyed meeting a lot of people from different countries (and the Netherlands), I learned to be more spontaneous and actually enjoy having free time, and I made friends for life. My experience abroad is one I will never forget.

Therapeutic poop, safe or not?

Demi van Dalen

The first medicinal use of poop was documented in the fourth century in China. Patients who were suffering from life threatening infections due to food poisoning, had to drink a golden soup of fecal material mixed in water. Later, in the sixteenth century, physicians used poop to treat other abdominal diseases as well. Nowadays, this treatment is officially known as fecal microbiota transplantation, or FMT.

Fecal microbiota transplantation

FMT is described as the transfer of fecal material from a healthy person into one suffering from a chronic gastrointestinal disease. Why would we do that? Under normal conditions, our gut is inhabited by commensal bacteria that help us protect and defend our intestines against infections. However, when our healthy microbiome is exposed to stressors like antibiotics, opioids, or an unhealthy diet, our health-promoting microbiota suffer and get replaced by a more pathogenic (unhealthy) community. In contrast with the healthy bacteria, the pathogens thrive in the changed environment and are able to outcompete the healthy microbes, thus producing the so called pathobiome.
Fecal microbiota transplantation with the feces of a healthy donor thus represents one of the promising treatment options to restore the healthy balance of the gut microbes. Research shows a correlation between a pathobiome and the occurrence of sepsis (blood poisoning). This suggests that FMT could also be an effective treatment for critically ill patients suffering from a pathogenic microbiome that is causing life-threatening sepsis. In fact, a recent a study demonstrated that FMT was beneficial in treating 2 patients with severe sepsis.

Mouse models of sepsis

Animal studies investigating FMT were performed on mouse models of sepsis in which mice received a pathogenic community via an injection into the body cavity (intraperitoneally). After this injection a FMT enema was administered and data showed that FMT saved the mice by clearing both the peritoneal and systemically disseminated bacteria therefore protecting the mice from death.
However, this model does not represent a spontaneous occurrence of gut-derived sepsis and therefore the laboratory of Dr. Alverdy has developed a model that includes all the factors that contribute to the occurrence of sepsis in human patients: western diet, opioids, antibiotics, surgical stress and lack of nutrition. This model is therefore more relevant to determine the effect of FMT on animals with sepsis.

In vivo experiment on mice

A total of 12 mice were used in our experiment. After eating the western diet for six weeks, the mice received 5 days of antibiotics and were fasted the evening before surgery. The mice were then randomly divided into 2 groups: the intervention group (PASH+FMT) and the control group (PASH).

To prepare the FMT enema, a healthy mouse from the same batch was sacrificed to harvest its cecum (a pouch that connects the small and large intestines). The cecal content was collected and a fecal solution was made by adding normal saline to a final concentration of 0.1 mg / mL saline. Within 2 hours after harvesting the cecal content, the FMT enema was delivered to the treatment group directly after surgery. Eighteen hours later the intervention group received another FMT enema. The control group received an enema with an autoclaved mix of FMT at the same time. The autoclave killed the bacteria in the solution and made the FMT ineffective.

FMT does not increase survival rate

Interestingly, while there was no statistical difference between the control and intervention group, the survival rate for the intervention group was lower (42,9%) than survival rate in the control group (50%). More mice died after receiving FMT. This is contradictory to our hypotheses and to the other studies with the initial sepsis mouse model.

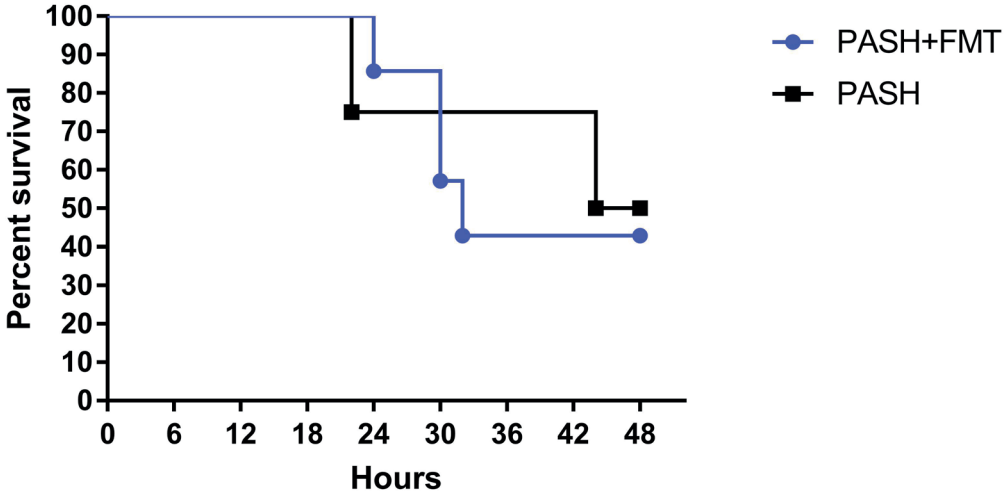


Figure 1. Survival curve

Bacteria and its metabolites

So why did the FMT not work in our intervention group? We hypothesized that the pathogenic bacteria in the guts of the sick mice may have made the gut barrier more permeable to bacteria, and that therefore the mice in the intervention group did not benefit from the FMT enema. After sacrificing the mice, we collected their blood, liver and spleen to check for bacterial dissemination in the blood and organs. However, we only found a difference in the amount of gram-negative bacteria present in the spleen of the intervention group.

Since we could not find significant differences in the bacterial dissemination data, we then hypothesized that it might not be the bacteria itself that somehow were influencing the body's reaction of the mouse, but the products of the bacteria present in the intestines of the sick mouse. The microbes in our gut, and in those of the mice, are important for the defense against potential pathogens. This is achieved not only by having the right, health-promoting bacteria in our intestines, but also through the products by those healthy bacteria, for example metabolites. Intestinal metabolites participate in various physiological processes like energy metabolism, cell to cell communication and host immunity. Short-chain fatty acids are key promotors of intestinal health and wall integrity. They are a source of nutrition for the cells in the intestinal wall, and play a role in reducing the risk of inflammatory diseases. Therefore, we decided to measure the concentration of those metabolites in the cecal content of the mice, as shown in figure 2.

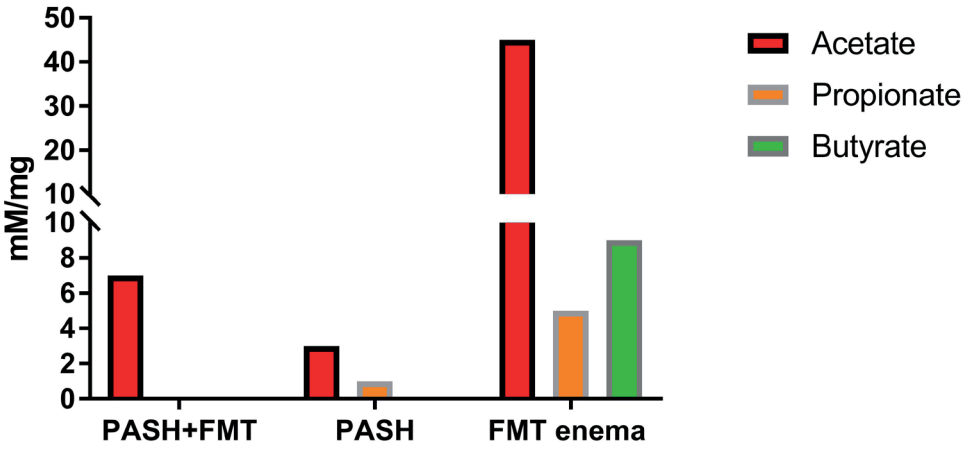


Figure 2. Cecal SCFA concentration

As is shown in figure 2, the FMT enema consisted of many more metabolites than both the cecal contents of the intervention and control group. The cecal content of the healthy donor mouse contained thus a high concentration of short-chain fatty acids. There was no difference in cecal SCFA concentration between the intervention and control group. So what could this imply? Well, based on the fact that healthy microbes produce these metabolites, this data suggests that FMT was not able to restore the microbiome of the intervention group and therefore its microbiome was not able to produce the same concentration metabolites as in healthy mice.

Future directions

This study investigated the potential role of fecal microbiota transplantation in a novel gut-derived sepsis model and its influence on the intestinal microbiome. This model incorporates several components that have a major impact on the microbiome of the mouse and are representable for the real situation of a critically ill patient on the ICU. These preliminary results suggest that this novel mouse model does not benefit from FMT. Because of the small sample size, we were not able to find significant results. It's therefore necessary to repeat this project with a larger sample size to determine the composition of the microbiome of the intervention group, and to decide whether the use of this therapeutic poop is safe for every patient.

Special thanks to
I would like to thank Dr. John Alverdy, and Olga Zaborina, for being great supervisors and supporting me during my internship. Special thanks Sanjiv Hyoju, Fons van den Berg and Emily Papazian for guiding me and teaching me the laboratory techniques I needed for my research projects. Finally, I want to thank my supervisor in the Netherlands, Prof. Harry van Goor, for linking me to this project and the inspiring lab in Chicago.

"My experience abroad gave me the ability to improve my confidence, social skills and English skills."

Rianne Damhuis

(Hengelo, 1996)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboudumc; The George Institute for Global Health, Sydney, Australia
Supervisors:	Prof. Dr. Karin Klijn, Floris Schreuder MSc; Prof. Dr. Craig Anderson, Dr. Xia Wang, Dr. Candice Delcourt

The Honours Programme gave me the opportunity to form a solid scientific base during my bachelor's degree. In the first year, my fellow students and I were able to have a close look at different research fields, where we focused on different subjects, each for a couple of weeks. During this time, I learned that neuroscience really appeals to me, but it also gave me a broader sense of all the different aspects of doing research.

In my summer internship, I started with a project in the department of Neurology, where I focused on blood pressure management for patients with brain haemorrhages. I didn't know a lot about doing research before, but the Honours Programme made it possible to work on a professional scientific level during my second Honours year.

As a cherry on the cake, I also had the opportunity to do my internship in the beautiful city of Sydney, Australia. The George Institute for Global Health was the place where I learned research skills related to my internship topic, but I also learned a lot from the other projects of fellow researchers. It was really impressive to see how the scientific work environment in another country really is. On top of that, my experience abroad gave me the ability to improve my confidence, social skills and English skills. These skills will be very useful in my future career, in which I definitely want to continue doing research.

On top of my personal growth, I am hopeful that the research I did with the amazing help of my supervisors will have a positive impact on patient care. I hope that with this research, healthcare professionals will be more aware of the importance of well-regulated blood pressure after an intracerebral haemorrhage, to prevent recurrent strokes in the future.

Put pressure on lowering blood pressure

Rianne Damhuis

Imagine you are in the prime of your life. Suddenly you are unable to speak and move your right arm and leg. Maybe it seems unlikely this will happen to you, but every year about forty thousand people in the Netherlands experience this; they get a stroke. About six thousand of them have bleeding in the brain, which is the most serious and disastrous form of stroke. Most of the time there is no clear underlying cause. In medical terms, we speak of a 'spontaneous intracerebral haemorrhage' (ICH).

Almost half of people with this condition pass away within the first five years after their ICH, and a lot of the survivors are disabled for the rest of their lives. Back to your imaginary situation. You survive your stroke. You are making small steps to recover, learning to use your body and to speak again. The last thing you want to happen at that point is another stroke to hit you.

Higher blood pressure, more recurrent strokes

Unfortunately, recurrences of intracerebral haemorrhages occur often. Different factors play a role in the likelihood of a recurrent ICH, such as genetics, the location of the bleeding, the use of anticoagulant drugs and having high blood pressure. Most of those factors are not easily changed, with the exception of blood pressure.

High blood pressure, in medical terms known as 'hypertension', in the months and years after the ICH, increases the risk of getting a recurrent ICH. Most of the people who have had an ICH, also have hypertension in their medical background. What is extra dangerous about hypertension is that people cannot feel if their blood pressure is high, so it is necessary to measure the blood pressure regularly to know if it is elevated.

Lowering the blood pressure is in most cases a safe and effective option to lower the risk of an ICH recurrence. The most recent guidelines therefore advise medical caregivers to control the blood pressure in patients who survive an ICH. Unfortunately, this does not always happen adequately in daily practice. Blood pressure is not always measured regularly and not every patient is getting the antihypertensive medication they need.

Predictors of poor blood pressure treatment

In the current research we tried to find out in which ICH patients the blood pressure was treated poorly on the 90th day after the ICH. 'Poor treatment' was divided in 'poor control', which was a blood pressure of at least 140 mmHg systolic, and 'poor management', which we defined as receiving no antihypertensive medication when the patient had a history of hypertension.

We used data from the 'Intensive Blood Pressure Reduction in Acute Cerebral Hemorrhage Trial' (INTERACT) 1 and 2. These studies are international randomized controlled trials designed to find out the effectiveness of 'acute intensive blood pressure lowering treatment' compared to 'guideline blood pressure lowering treatment' in the first hours after the ICH. However, we focused on the long term blood pressure data. In total, 3,233 patients who had an ICH were included. There was data on the three months after the ICH, including the measured blood pressure for 348 patients, and whether the patient used blood pressure lowering medication for 2,819 patients (of whom 2,045 with a history of hypertension). We also had (medical) background information of these patients. The patients in this study were from 21 different countries, but most of them (71%) were recruited in China.

We looked for factors that predicted if someone had high blood pressure three months after their ICH ('poor blood pressure control'). We did this using the statistical test called 'binary logistic regression'. This analysis can find factors that predict an outcome, in this case predictors of high blood pressure (systolic blood pressure of 140 mmHg of higher). We did not find any predictors of high blood pressure ('poor blood pressure control') at three months after the ICH. This might be because we only had actual blood pressure data at this time point in 348 patients.

However, we did have data on the use of blood pressure lowering medication at three months after the ICH in most patients. So we also looked for predictors of 'poor blood pressure management'. If a patient did not get blood pressure medication at day 90 although he or she needed it, we defined this as 'poor blood pressure management'. We selected all patients with a history of hypertension (because they were most likely to need medication) and looked for predictors of not receiving blood pressure lowering medication three months after the ICH. Again we used the statistical test binary logistic regression.

With this analysis we found five predictors of poor blood pressure management: being 65 years or older, having joined the INTERACT study in China, not being assigned to the intensive blood pressure lowering treatment in the acute stadium (which means receiving the non-intensive guideline treatment in the first hours after the ICH), having a low consciousness on the day of the ICH (Glasgow Coma Scale lower than 13), and having a blood pressure lower than 180 mmHg systolic on the day of the ICH. This means that people with one or more of these conditions get the medication they need less frequently compared to people that do not have these conditions. For example, patients aged 65 years or older are more likely not to be receiving necessary medication than younger patients. Furthermore, the blood pressure of patients from China does not seem to be managed as well as from patients from other countries.

Recommendation

Based on the above results, we strongly believe there are five predictors of having poor blood pressure management. Caregivers should therefore give extra attention on achieving an adequate blood pressure, and delivering suitable blood pressure medication for these ICH patients. This will therefore reduce their risk of a recurrent stroke.

However, most of the patients in this research were recruited in China, and because both ethnicity and healthcare systems can play a role in the blood pressure control, we are not sure whether we can implement our results in the Dutch healthcare system. Furthermore, we do not have data from the Netherlands on how many ICH patients do not get the necessary blood pressure measurements, have high blood pressure, or do not receive the medication they need.

What is next?

Because of these factors, this research needs to be continued in the Netherlands. We are therefore conducting research on this topic, in a study on ICH patients admitted to the Radboudumc and registered by general practitioners around Nijmegen. We are following these patients in the years after their ICH and counting the number of times they get a blood pressure measurement. In addition, the actual blood pressure and use of blood pressure medication are being tracked. Thus we can investigate the current status of the blood pressure control and management of these patients. Our final aim is to better manage and control the blood pressure in all patients surviving an intracerebral haemorrhage, to lower the chance of recurrences, so that patients can focus on their recovery.

Acknowledgements
I am really blessed to be supervised by three amazing and closely involved daily supervisors, Floris Schreuder MSc at the Radboudumc and Dr. Xia Wang and Dr. Candice Delcourt in The George Institute for Global Health in Australia. Furthermore, I would like to thank Prof. Dr. Karin Klijn from the Netherlands and Prof. Dr. Craig Anderson from The George Institute for their great support and feedback, and for allowing me to do this research at these institutes.

Stefan van Dinter

(Nijmegen, 1997)
Bachelor of Medicine, 2015-2018

Honours Internship:	Department of Cardiology, Radboudumc; Department of Laboratory Medicine, San Francisco General Hospital, University of California, San Francisco, USA
Supervisors:	Dr. Marc Brouwer, Drs. Etienne Cramer; Prof. Dr. Alan Wu

For me, the Radboud Honours Programme Medical Sciences was by far the most wonderful, educational, if not most challenging period of my career so far. Without intending to make this collection of words look too much like a sales advertisement for this programme, I would definitely say that it has been a great contribution to my professional development. Personally, I've always been someone who found it very difficult to step out of my comfort zone, but with the help of the Honours Programme I managed to overcome this characteristic which had been limiting my potential ever since high school.

As a medical student you basically look up to doctors, and it was an honour to be able to work closely with two cardiologists, Dr. Marc Brouwer and Drs. Etienne Cramer, who have contributed a lot to the academic field. To approach those two men and convince them that I was worth investing time in wasn't easy at first, but after a tough start they've provided me with more knowledge than I could have imagined, as well as an internship which I wouldn't have exchanged for anything else.

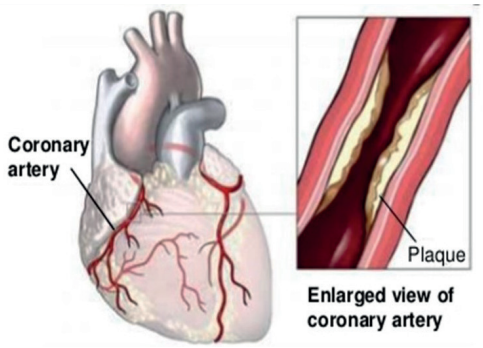
I got offered an internship with the San Francisco General Hospital at the University of California, San Francisco. First of all, take away the price tags, and I believe I got a chance to visit one of the craziest, most tremendous cities in the world. On top of that, being able to use state-of-the-art techniques, getting involved in game-changing studies and being supervised by a renowned scientist in the academic world, Prof. Dr. Alan Wu, was all I could ever hope for.

In summary, this programme has been a huge contribution to my development and has definitely unveiled opportunities for me in the nearby future.

One step ahead of a heart attack with just a drop of blood

Stefan van Dinter

With cardiovascular diseases currently being the leading cause of mortality, accounting for 31% of global death, they have become a popular topic of everyday discussions and a major point of concern for human health. Additionally, with the 17.7 million deaths per year caused by cardiovascular diseases expected to increase to an astonishing 23.6 million, new treatments must be discovered soon. However, cardiovascular diseases do not have a well-defined single cause and even though doctors and researchers are trying their very best to reduce the disease incidence, conditions such as diabetes or the seemingly inevitable rising trend in dietary salt, sugar and fat intake have made it very difficult to reduce disease risk.



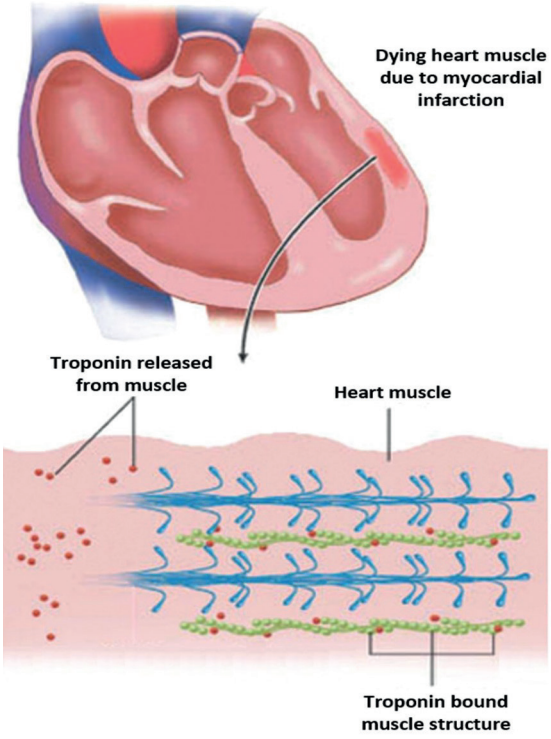
Cardiovascular diseases consist of multiple conditions related to either the heart, blood vessels or both. The most common cardiovascular disease is myocardial infarction, or a 'heart attack', typically caused by partial or complete blockage of the vessels of the heart (see figure 1). In this case, blood flow will be compromised and the heart muscle is left with a shortage of nutrients. As a result, the muscle will slowly die if the situation is not promptly detected and reversed. In other words, 'time is muscle'.

Figure 1. Schematic picture of a partially blocked heart vessel (coronary artery)

From big picture to small details

Now picture a patient coming in the emergency department with chest pain. In this case, three pieces of information essentially build the diagnosis: an electrocardiogram (ECG), the measurement of troponin levels and the collection of patient symptoms. Let us look at troponin more closely. It is a protein which is present in every muscle, attached to all muscle cell components to regulate contraction and relaxation. However, there is a special type of troponin only found in the heart muscle called cardiac troponin. Within this, two unique proteins have been found so far: cardiac troponin T (cTnT) and I (cTnI). This article focuses on the latter, although both cTnT and cTnI share similarities and can be used for diagnostic purposes.

In a situation where the heart muscle is slowly dying such as in the case of a myocardial infarction, troponin is detached from the muscle (as shown in figure 2) and released in the blood, where it can be detected by lab tests. In most cases the more severe the area



of infarction is, the higher the observed rise in troponin, leading to a more obvious diagnosis. Nonetheless, in some cases, especially when coronary vessels are partially blocked instead of fully occluded, the troponin increase can be relatively small at first. Keeping in mind that diseases like kidney failure or COPD (a disease frequently seen in extensive smokers) often also cause small elevations of troponin, confusing situations occur in which it can be extremely difficult to deduce from cardiac troponin whether an infarction is present or not.

Figure 2. Troponin, normally bound to the muscle structure, is released to the blood in the situation of a myocardial infarction

Time to improve troponin testing

The cardiac troponin rise in non-heart diseases has led to many research questions. Should we look for an alternative for troponin? Should we use additional markers? Is there a way to improve present-day troponin tests? Current modern technologies, such as mass spectrometry, have given rise to new possibilities with the potential to improve troponin testing. For the past twenty years, we have mainly been looking at troponin as a freely circulating, heart muscle-specific protein released during cardiac damage. However, novel approaches are changing the research perspective by investigating new, different forms of cardiac troponin.

Fragments and vesicles

An increasingly popular detection technique is mass spectrometry, capable of detecting and distinguishing between the smallest particles by using molecule-specific characteristics such as size and charge. Larger troponin fragments have already been detected by simpler methods and are known to be prominent in certain diseases. Since the smaller fragments are very difficult to detect, due to their instability and being unable to be captured by our current clinical tests (see the green, Y-shaped antibody in figure 3) their existence has not yet been confirmed. However, because of their small size, smaller fragments could potentially 'leak' out of cells earlier than the bigger fragments. Therefore, being able to detect them could be extremely beneficial in early disease diagnosis.

By making use of state-of-the-art mass spectrometry, we found that we were able to track down small, custom made particles that precisely mimicked small troponin fragments. This new information could be used to set up a useful technique for early diagnosis, since this verifies the capability of mass spectrometry to detect the small fragments. However, in contrast to our own custom-made solution, the suspected amount of small troponin fragments in human blood is so low, that unfortunately current mass spectrometers are not sensitive enough to detect them yet. Nonetheless, it is expected that this will be possible in the near future, when techniques are even more advanced.

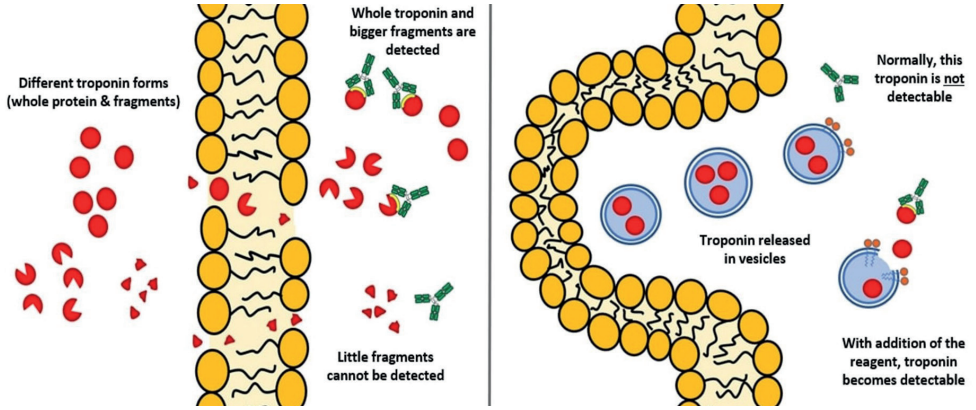


Figure 3. An overview of the hypothetical ways of troponin release. On the left, troponin release is depicted as migration through the cell membrane as intact proteins, big fragments or small fragments. The detection by antibodies is also made visible. On the right, the release of vesicles containing troponin is shown and the troponin-releasing effect of adding a detergent (orange dot with blue tail).

Whereas small troponin fragments might still be impossible to detect, a different aspect of troponin is already easily detectable with modern techniques. This aspect, which currently is one of the 'hot topics' in the academic world, is the existence of extracellular vesicles. These vesicles are essentially cargo-carrying bubbles that have separated themselves from cells and contain some of the cell content. Not only does the amount of vesicles released into the bloodstream differ between various diseases but also the content they are carrying. Because the coat of these vesicles is tough, their cargo is protected from the outer environment, making it impossible to reach with detection methods.

Recently, researchers started wondering whether troponin could be residing in these vesicles, and it was necessary to break the vesicle wall to find out. We used a reagent known to be capable of breaking membranes and releasing the vesicle content (see figure 3), and by doing so, we were the first to find evidence that this previously unattainable portion of troponin is indeed existent in microvesicles.

Future approaches

The next step is to closely examine the clinical applicability of our discoveries. With regard to the small fragments, it is highly encouraging that we have found a mass spectrometry protocol to detect them. However, we are dependent on more advanced spectrometers if we want a chance to detect them in real human samples. The knowledge that troponin resides in vesicles is closer to being used in clinical testing. However,

more understanding is needed about the different amounts of troponin from vesicles in different diseases. In summary, both discoveries open up endless research opportunities and, perhaps by the time technology and our knowledge have advanced, we will be ready to face cardiovascular diseases and counteract its huge burden on global health.

Acknowledgments
I would like to give my special thanks to my supervisors, Dr. Marc Brouwer, Drs. Etienne Cramer and Prof.Dr. Alan Wu, who have taught me many new, valuable things and gave me countless opportunities. I also wish to thank my coach, Dr. Desireé Oosterbaan, my parents and other family and friends for their support throughout both easy and tough times.

"Being able to attend the surgical procedures and subsequently interview all those patients (...) has been very special and gave me a deep understanding about the importance of doing research."

Pauline Groenen

(Venray, 1996)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Anatomy, Radboudumc; The Spine & Pain Institute of New York, New York, USA
Supervisors:	Prof. Dr. Kris Vissers, Dylan Henssen Msc; Dr. Kenneth B. Chapman

I have always had a great interest in doing research: being able to contribute to the knowledge of the human body, diseases, and possible treatments always fascinated me. The Honours Programme Medical Sciences offered me the extra challenge I was longing for and gave me the opportunity to gain more insight into the variety of biomedical research themes and the ways research is conducted.

Due to my special passion for neuroanatomy, I started the individual part of the programme at the Department of Anatomy by helping to set up an experimental study which explored the use and efficacy of neuromodulation for patients with trigeminal neuralgia. In addition to the research skills and extensive neuroanatomical knowledge I gained, the most important lessons I learned from this internship were how to set up a research study, and how to persevere during the many setbacks that come along with this.

To live in New York City, the city where everything 'happens', has always been a dream for me. To arrange this internship has been a huge challenge, but it was definitely worth it. At the Spine & Pain Institute, I had the opportunity to set up an experimental research study with the state-of-the-art treatment 'dorsal root ganglion stimulation' in patients with low back pain. Being able to attend the surgical procedures and subsequently interview all those patients, and hear them explain the huge impact this DRG-stimulator had on their lives, has been very special and gave me a deep understanding about the importance of doing research. Moreover, I got the opportunity to help writing three technical reports, a hypothesis paper (all awaiting publication) and to attend a conference in Miami. New York has been a once in a lifetime experience that really changed my life.

Life-changing treatment option for chronic low back pain patients

Pauline Groenen

'The following items are about activities you might do during a typical day. Does your health now limit you in these activities?' This is a standard question, part of the SF-36 questionnaire, which I asked a 44-year old female patient before she went to the operating theatre to receive the dorsal root ganglion stimulator to treat her chronic low back pain. 'Oh dear, if only you knew...', she answered me, with tears welling up in her eyes. 'I used to be a nurse, but right now, I am not able to carry groceries, push a vacuum cleaner or even wash or bathe myself anymore. I need help in almost every aspect of my life because of the severe and extreme pain I experience'. Afterwards, the patient told me that she was glad that Dr. Chapman took her complaints seriously, that this treatment was felt to be the last resort to treat her pain, and that she hoped to be able tell me she was doing better in two weeks, during the appointment in which we would meet again.

Chronic Low Back Pain

Low back pain affects many people worldwide and is one of the top five most common physician visits in the United States. In thirty percent of these patients, the pain persists for more than one year and results in substantial limitations in daily life activities. Although so many people are suffering from low back pain, in most of the cases, the cause remains unknown. This makes low back pain a difficult disease to treat. Spinal cord stimulation (SCS) has emerged as a possible treatment option to treat chronic and untreatable cases of this condition. Although SCS has shown to be effective in some patients, in others the pain remains or comes back after a couple of months. More recently, dorsal root ganglion stimulation (DRG-stim) has emerged as a further possible treatment option.

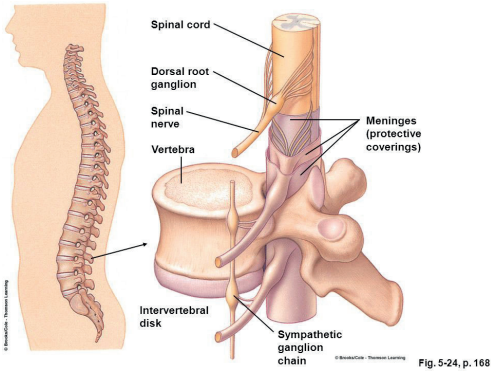


Figure 1. At each vertebral level, two dorsal root ganglions enter the spinal cord. A dorsal root ganglion contains the sensational information of the specific body area (dermatome) it corresponds to.

Spinal cord stimulation vs. Dorsal Root Ganglion Stimulation

The spinal cord is positioned in the vertebral column and contains the nerves that are connected to all parts of the body. The specific nerves that carry information about the pain or nociception of a certain area, for example, the low back, enter the spinal cord at a certain position. However, before the pain nerves of the low back merge with the spinal cord, they first enter a center in which many nerves carrying information about sensations are concentrated. This center is called the 'dorsal root ganglion' and lies just next to the spinal cord (see picture). In contrast, the nerves responsible for movement leave the spinal cord via the ventral root. After leaving the dorsal root ganglion, sensational signals enter the dorsal column of the spinal cord. The dorsal column is the part of the spinal cord in which sensational signals travel towards the brain. This is the part which is targeted with conventional spinal cord stimulation.

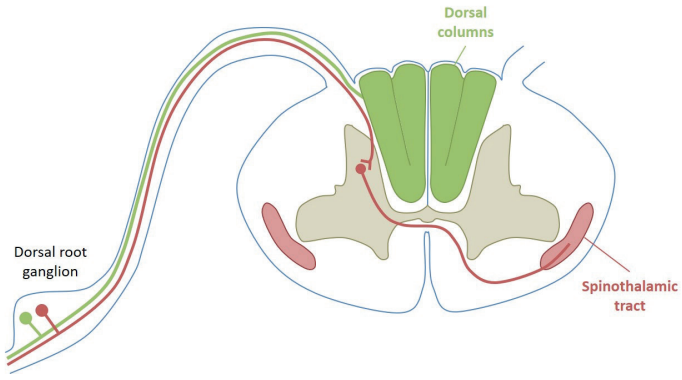


Figure 2. In red = nociception. In green = proprioception, vibration and fine touch.

What makes DRG-stim a winner?

There are four major reasons why Dorsal Root Ganglion-stimulation (DRG-stim) is a better treatment option than stimulation of the dorsal column:

- **Position**
  Due to its position just outside the vertebral column, the DRG is an easier target for treatment. In addition, this results in a less dangerous surgical procedure, since the spinal cord itself is not involved.
- **More specific**
  The DRG only contains sensational information about a limited body area (dermatome) such as the low back. Stimulating the DRG is therefore more specific. By targeting the dorsal column of the spinal cord at a certain position (SCS), the nerves that enter the spinal cord at that position and all the nerves that have entered below this position are stimulated. SCS is stimulating the entire tract (medial lemniscal system) instead of a single nerve. This means that an entire body area is stimulated rather than a specific location.
- **Pain is tackled**
  All sensational information travels through the DRG before entering the spinal cord. However, nociceptive information is the only sensational entity that does not enter the

dorsal column of the spinal cord but crosses over to the contralateral side to enter the spinothalamic tract. This fact makes stimulating the dorsal column of the spinal cord SCS more questionable since it does not affect the pain signal pathways.

- **Less side effects**
  SCS does stimulate the sensational pathways responsible for fine touch and vibration, which does lead to side-effects like tingling and numbness. On the contrary, the DRG contains nociceptive information of a specific body area which makes it a more direct and precise target with less side-effects.

Results of DRG stimulation in 14 patients with low back pain

We performed a study in which we treated 14 patients with chronic low back pain with a dorsal root ganglion stimulator. At baseline, all patients reported severe disability both physically and emotionally, which interfered substantially with their daily life. Treatment with DRG stimulation was considered the last resort to treat their pain. The visual analog scale (VAS) was used to measure their pain, with 0 meaning 'no pain' and 10 meaning 'the worst pain imaginable'. At an average of 3.5 months follow-up, the VAS scores improved significantly from a median of 9.1 at baseline to a median of 1.7 at follow-up. More than 75% of the patients experienced a pain relief of 80% or more. Moreover, we used several questionnaires to measure quality of life and physical and mental functioning which also showed a great and significant improvement. These outstanding results therefore made a huge difference to the patients' lives.

What is our secret?

Other studies that used DRG stimulation to treat low back pain showed approximately 50% pain relief in their patients. Compared to these, our results with an average pain relief of 90% are almost unbelievable. How is that possible? The big difference is that we used an alternative vertebral level to perform DRG stimulation. Traditionally, the dorsal root ganglion is coupled to the dermatomal map: a specific body area or dermatome is innervated by the spinal nerve and DRG of a specific vertebral level. In this way, the low back corresponds to L4-L5. This is a logical way to decide to implant the DRG stimulator at the DRG that corresponds to the low back dermatome. However, by using a trial-and-error method in patients we found out that we got a better coverage of the low back pain by stimulating the DRG of a higher vertebral level. We have a theory that the traditional anatomical view on the low back pain pathways are inaccurate and that in the dorsal horn of the spinal cord ascending and descending pain tracts exist that ultimately converge at a particular vertebral level. This vertebral level lies way higher than where the original dorsal root ganglion entered the spinal cord! However, this is just a theory, and more fundamental research into the low back pain pathways needs to be performed to find out what exactly happens in the backs of our patients.

Our 44-year old female patient two weeks later

Two weeks later I met the same 44-year old female patient to fill in the same questionnaires together. She was walking into the room, something she hadn't done in three years. She was smiling happily, and she told me she 'had never felt so good in the last three years'. The pain has vanished: I can walk, I can dance, I even went to a wedding party this weekend, and right now I want to kiss Dr. Chapman', she told me. She became happier with every question I asked her because she was remembering what she had answered two weeks before. She was crying again, but this time she didn't cry because of pain, it was tears of joy because she was finally able to get her old life back.

Special thanks to
Prof. Dr. Kris Vissers, Drs. Dylan Henssen and Drs. Hans Timmerman for teaching me so much about conducting research and supporting me to find a great research internship abroad. Dr. Noud van Helmond for teaching me about statistics and helping me write reports that may be published in renowned journals. Dr. Kenneth B. Chapman for hosting me in New York, for his enthusiasm and passion for treating patients and conducting research that has a direct impact on patients' lives, and of course for the great opportunities and experiences he provided me. I would also like to thank the patient that allowed me to use her story in this article.

"Although we experienced several minor setbacks during my internship with the project, I am very satisfied with the results and my own growth in research skills."

Wibe Hoefsloot

(Zutphen, 1997)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Anatomy, Radboudumc; Dr. Robert Chen's Lab, Department of Neurology, University Health Network, Toronto Western Hospital, Toronto, Canada
Supervisors:	Dylan Henssen, MD; Prof. Dr. Robert E.W. Chen

The Honours Programme has given me the opportunity to explore the field of clinical research both at the Radboudumc in Nijmegen and abroad. Before starting this journey, I didn't have any real experience with performing research in a clinical setting, but now I have successfully worked on two different studies during my honours internships!

My internship in Nijmegen at the department of Anatomy under the supervision of Dylan Henssen broadened my understanding of neuroanatomy, neurophysiology and anaesthesiology. Starting as a rather unexperienced research student, I developed many lab skills and got a good grasp of clinical research. My study involved a special brain stimulation technique called TMS (Transcranial Magnetic Stimulation) and with the help of the Donders Institute for Brain, Cognition and Behaviour, I learned to perform this technique myself. I found it quite fascinating how this could stimulate various areas of the brain to achieve positive results used for the treatment of depression, movement disorders and chronic pain. Although we experienced several minor setbacks during my internship with the project, I am very satisfied with the results and my own growth in research skills.

My internship abroad started in Toronto, Canada, at Dr. Robert Chen's research lab at the Toronto Western Hospital. I had the opportunity to live in an astonishing and massive city, meet new international friends and extend my personal development. My project in Canada was related to my study at the Radboudumc and was based upon this same brain stimulation technique. However, it focused more on the exact mechanisms underlying the clinical results. Attending our regular lab meetings, my colleagues and I were able to strengthen the research projects and increase our critical thinking. I had some great experiences in Canada and it was one of the best periods of my life!

Treating pain by zapping the brain

Wibe Hoefsloot

"I even returned to work!" This is just one of the quotes from participants in a new study investigating brain stimulation in chronic neuropathic pain patients. These patients were all hampered by this condition in their daily lives, but not long after the experiments they experienced less pain and an improvement in their quality of life. In my particular study, we further examined a technique called transcranial magnetic stimulation (TMS), which is used to stimulate the brain for outcomes such as pain relief.

Mechanics of TMS

But what is TMS exactly? TMS is an indirect way of stimulating the brain, without any invasive actions. This is in contrast to deep brain stimulation that is frequently in the news for the treatment of Parkinson's disease, which involves surgically opening the skull and placing an electrode into the brain. In TMS, a device is simply put against the head over the location that requires stimulation. By activating the machine, an electric current will go through a metal coil and this will create a magnetic field around it, which will in turn create an impulse in the brain cells. Combining multiple pulses into a protocol with a specific frequency, strength and orientation, a wide variety of possibilities exists to use this technique and this is called repetitive TMS (rTMS).

Besides the different settings of the TMS machine, the location is also of great importance to achieve the desired effect. The TMS pulse only stimulates a small area, approximately 1 cubic centimetre. Every location in the brain is directed to do a certain task. It can be quite challenging to find these locations, since it isn't possible to simply look at it through the skull. Basic measurements are sometimes taken using common anatomical landmarks to make an estimation. More accuracy is gained by using an MRI scan combined with the coil position using special computer software. Stimulating the motor area is slightly easier, since a strong enough stimulus will create a movement in the corresponding muscle controlled by that brain area.

Clinical practice

TMS is already used in clinics to treat several diseases. Most renowned is the treatment of depression with repetitive TMS. Patients suffering from major depression where all other medicine is ineffective are eligible for rTMS. Nowadays, a different technique called electroconvulsive therapy is the standard for patients not responding to conventional medicine, but this could be seen as a rather uncivilized method since the patient is actively brought into an epileptic insult. However, performing rTMS on the frontal part of the brain with a high frequency, repeated over a number of days can have a positive effect in the treatment of depression as an alternative for electroconvulsive therapy.
Besides depression, rTMS is accepted as a therapy for other mental illnesses as bipolar disorder, obsessive compulsive disorders (OCD) and Post Traumatic Stress Disorder

(PTSD). However, widespread usage of the technique in the clinic still lacks foothold. TMS can also be applied as a diagnostic tool evaluating specific brain pathways. In patients with stroke, TMS can help evaluate damage to the motor pathways by measuring the time it takes for the signal to reach the muscle from the brain and the strength of the muscle twitch made by the signal. This could of course also be used for other damaging neural diseases, for example Multiple Sclerosis, Amyotrophic Lateral Sclerosis, and neuronal disorders affecting the nerves responsible for vision, hearing and many other functions.

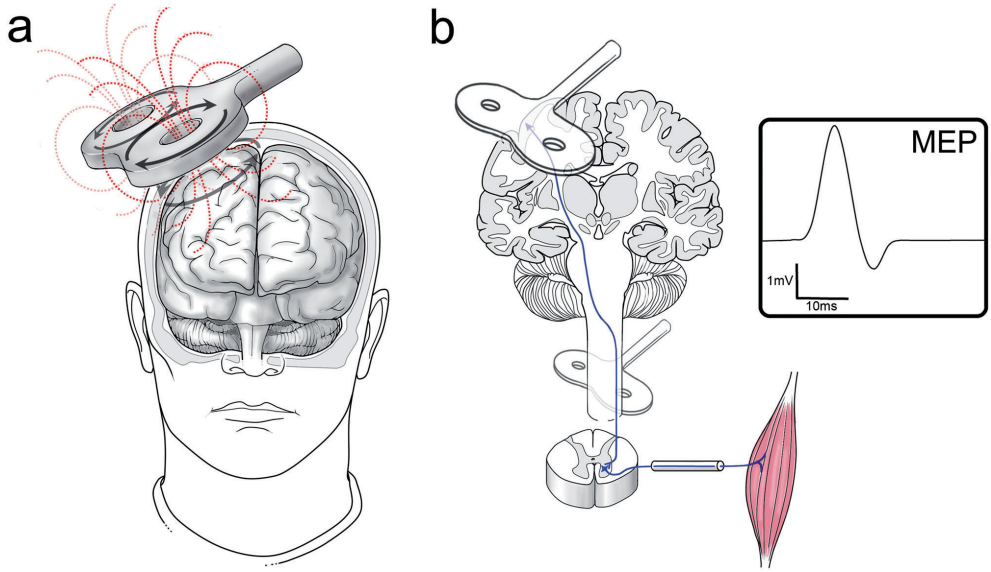


Figure 1. Transcranial Magnetic Stimulation (TMS).
a: A visualisation of TMS of the brain.
b: Stimulation of the motor area creating a pulse towards the controlled muscle resulting in a muscle contraction. (Reference: Vlachos, A., Funke, K., & Ziemann, U. (2017). Assessment and modulation of cortical inhibition using transcranial magnetic stimulation. E-Neuroforum.)

TMS and chronic pain

Around the year 2000, researchers started to use rTMS as an alternative for epidural motor cortex stimulation, which was already used for about a decade to treat unresponsive chronic pain. Epidural motor cortex stimulation showed promising results. However, for this technique a surgical operation is necessary, similar to deep brain stimulation mentioned before in Parkinson's Disease. This is quite an invasive operation and TMS could be an alternative treatment, resulting in an easier procedure without any surgery. It could also be used as a way to indicate the effectiveness of epidural motor cortex stimulation, since this technique does not work as effectively for everyone.

The past two decades, much research has been done to evaluate the effectiveness of rTMS on chronic pain. It is quite difficult to find all the best settings. Frequency, position, orientation of the device, number of stimuli, stimulus strength, amount of pulses given at once and the amount of sessions all have some influence on the total effect of the treatment. Therefore, a consensus is still missing on all the parameters, and major

studies with more participants are also lacking.
There are some general guidelines now described in the literature. Only a high-frequency protocol (more than five times per second) induces the positive effect. Furthermore, the stimulus strength should be around ninety percent of the minimal threshold to get a muscle twitch. Lastly, the device should be held at a position parallel to the midline of the head focusing on the hand motor area.

The treatment seems to work best in patients with orofacial neuropathic pain, which means that the sensory nerves in their face are not working as they should because they are affected by neural diseases or were damaged, for example, during an operation. These patients are severely incapacitated and their quality of life is hampered. Other pain disorders, such as fibromyalgia and complex regional pain syndrome, have also been studied but show less encouraging results.

Stimulation of both sides of the head

Although there is still no consensus on the stimulation protocol, there are more and more promising studies out there and the mechanisms are better understood. In my study, we compared dual stimulation of both sides of the head with the conventional stimulation of only one side of the head in chronic neuropathic pain patients. We theorized that stimulating both sides of the head at the motor area would have an increased effect in pain relief.

The stimulation protocol we used was a frequency of 10 times a second in a burst of 10 seconds, followed by a pause for 50 seconds. This was repeated 10 times to a total of 1000 pulses for a side. The target site would be the hand motor area, which would correspond to previous studies. The stimulation strength followed the present guidelines mentioned above.

The patients in our study all suffered from orofacial neuropathic pain. The patients visited the lab twice, once where they were treated with one-sided stimulation and the once with both sides of the head. In between the visits, there was at least one week so the possible effects of the first stimulation settings had worn off. The days before and after the stimulation sessions, the patients filled in an elaborate questionnaire describing their pain on multiple dimensions for the research team to have a thorough understanding of their pain. In addition, they gave their pain level a number ranging from 0 to 10, with 0 as no pain up to 10 as the most horrible pain ever. These results could be compared between the different stimulation sessions and their baseline values.

Patients reported that their pain was more easily managed after the dual stimulation compared to the conventional single stimulation, but more importantly, they experienced an improvement in their quality of life! They participated in more social activities, had more energy in their daily life and felt overall better after the stimulation. This could perhaps be a new discovery further opening the doors to widespread pain treatment.

Conclusion

While our study is an ongoing one, even more research must be done before TMS can be used to treat chronic neuropathic pain in a more public setting. In the United States and Canada, TMS is already sporadically used as a treatment for this patient group, but

throughout the rest of the world, it is still only in the experimental stages. With our own research, we hope to have made a new step towards the next generation pain treatment. TMS seems to be a very promising treatment and hopefully it can be an option for more patients in the future.

Special thanks to
Dylan Henssen for his teaching and dedication for this project and helping me be part of it. Prof. Dr. Robert E.W. Chen for his guidance and support during my internship in Canada at the Toronto Western Hospital. Dennis Schutter for his help with performing the experiments and teaching me how to handle the TMS device and do the procedures. My lab members in Canada for their help and friendship during my time there.

"The honours programme has shown me how crucial research is in my field as a future dentist. It has allowed me to get hands-on experiences and find out how fascinating it can be."

Zjenja Jegorov

(Sint-Petersburg, 1997)
Bachelor Dentistry 2015-2018

Honours internship: Department of Biomaterials, Radboudumc, Nijmegen
Supervisors: Prof. Dr. J.A. Jansen, Dr. X. Frank Walboomers

I decided to join the honours programme to gain more experience in medical research and to challenge myself. As a dentistry student you mainly focus on patient care; research is not given much attention. The honours programme has shown me how crucial research is in my field as a future dentist. It has allowed me to get hands-on experiences and find out how fascinating it can be. It has expanded my horizon and given me opportunities that I am very grateful for.

Another thing my research has shown me is the great possibilities for practical implementation. Tissue engineering is still very innovative and not often used in practice yet. Our ultimate goal is to form new teeth from stem cells, so in the future, when this process can finally can be applied in the dental chair, it will feel like the sky is the limit! For my internship abroad I was to go to Boston. Due to circumstances outside my control I was not able to go. Nevertheless I had a great educational experience in Nijmegen. I arranged to transport my cells from Boston to Nijmegen and set up a whole new research project with my supervisor. In the beginning, it felt like trial and error, which was frustrating at times. But research is never straightforward. Often you will not get the results you would have wanted. Of course this does not mean that your work is meaningless; this was something that I have realized throughout my research. Overall, I have also learned a lot regarding organizational and logistical matters.

To conclude, I have enjoyed the honours programme. Besides conducting research, I have made new friends and gained life experience! I would definitely recommend it to everyone!

Because of the delay caused by his not being able to go to the USA, the deadline for this book came too early for Zjenja to contribute an article about his research.

"During the program, my fellow students became real friends. As busy as we were, we could always find support within our group."

Claire Koeyvoets

(Sittard, 1997)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Genetics, Radboudumc; The Mind Research Network, Albuquerque, New Mexico, USA
Supervisors:	Dr. Alejandro Arias Vasquez; Dr. Jingyu Liu

I saw the honours programme as a chance to gain more knowledge about research and meet researchers in different fields, but it turned out to be much more than that.
Being interested in psychiatry I chose to do my internship on ADHD (Attention Deficit Hyperactivity Disorder). I learned a lot during my research. Working with computer scientists and mathematicians, I found myself to be completely out of my comfort zone. They taught me about mathematics and programming and I taught them about ADHD, brain structures and genetics. This way I felt like an important addition to the team. Finding results was a good challenge, and this made me grow as a researcher and person. It taught me to be more persistent, and it made me even happier when I finally did have some results.

As a real city girl, I could not picture myself staying in the natural environment of New Mexico. And Albuquerque is not really a big city itself. However, after I found myself camping for 4 days without a tent and living on instant noodles, I can say that these camping trips were, unexpectedly, some of the best experiences I had during my time. I fell in love with Albuquerque and New Mexico and I am glad that I spent my internship at this beautiful location.

During the program, my fellow students became real friends. As busy as we were, we could always find support within our group. And sometimes we ended up in a bar drinking beer after one of the periods of hard work.
I met amazing people, worked with a great team and liked my research a lot. Everybody made me feel welcome and, in difficult times, I had good friends for support. If I were able to go back in time, I would do it all again.

How does genetics affect the brain in patients with ADHD?

Claire Koeyvoets

Many of you probably had this kid in your class in primary school that was always busy. Always running around, unable to sit on his/her chair and pay attention. Soon you learned that these kids were not just "Alle Dagen Heel Druk" (every day, very busy), but they had a disease called Attention-Deficit/Hyperactivity Disorder, or ADHD. General knowledge about ADHD is that it is a childhood disease, and only a few people know that 50-75% of these children with ADHD will be affected by this disease throughout their entire life. Most of these children experience a decrease in hyperactivity symptoms, but the inattention symptoms remain a problem. Unfortunately not much can currently be done for people with ADHD because the knowledge about this disease, its genetics, its brain differences and the interaction between genetics and the brain is still quite limited.

Mathematics for Psychiatry

Nowadays there are not many suitable methods for investigating the interaction between genetics and brain differences in ADHD, which contributes to the lack of research in this field. In our research, we tried to find this interaction using a parallel independent component analysis, based on mathematics. Imagine that you are at a cocktail party and all the guests are talking. There is no way to tell which sentences belong to one single person. Using this analysis method you are able to separate all the different voices into independent components (sources or in this case people). The analysis does the same with genetics and structural brain images. The program sees which genetic parts belong together and puts them together in one component. It does the same with the brain regions that belong together. This way, the result is separate genetic and brain components. Afterwards, the program matches the genetic components with the brain components to find the best match possible. This gives specific genetic data which is related to specific brain regions.

So, what genetics do we need?

Our DNA is so complex, that it is not possible to use all of it to perform the analyses. Our DNA contains our genes and our genes are build out of base pairs, which are a pairs of nucleotides. Therefore, nucleotides are single spots at a specific location in your DNA. Variations in which nucleotide is in such a spot are called single nucleotide polymorphisms (SNP). We decided to use a couple of thousands of those SNPs for this research (from 4500 to 8900 SNPs, ranging over different sets). Earlier research had already identified the SNPs that have the highest correlation to ADHD and based on this knowledge we selected the genetic information. By using this method we had SNPs in our analysis that should be involved in ADHD.

Information overload

A few thousand SNPs is still a lot of information for the program. From previous research, we knew which mechanisms in the brain might cause ADHD. These different mechanisms are called pathways. We then selected SNPs involved in a few pathways. These are two neurotransmitters, gamma-aminobutyric acid (GABA) and dopamine, which are chemicals that make the transport of information possible. Furthermore, we selected nervous system development, neurotransmitter release and clearance, inflammation, signal transduction. The SNPs in these pathways were added to the program, telling it that it needed to pay attention to those SNPs because they are important. This way it is easier for the program not to get lost in the amount of genetic information by focusing on a smaller part.

Questioning our existing knowledge

After running the analysis with the references that should be involved in ADHD, we did not get the results we were hoping for. We found genetic parts that were related to brain regions, but they are not involved in ADHD. Both the genetic part and the brain region were the same in patients with ADHD and healthy controls. In addition, the pathways we added did not show a difference in patients and controls, which might indicate that these are not (solely) involved in the existence of ADHD. Furthermore, the pathways did not seem to affect any brain regions. Adding the pathways did not work the way we expected, perhaps because our prior knowledge is not entirely correct or because there is a lack of important information. This indicates that we need to do more research into how ADHD works and what really happens in the brains of the patients.

Adding more information

Since we found out that limiting the amount of information by using less genetic data or by using a reference did not give us results, we decided to increase the amount of information. We increased the study population by re-adding family members of participants. Furthermore, we increased the amount of SNPs from 8900 to 13.000 to 30.000. We provided the program with this information to see if it could create more consistent results.

It will all be a forgotten memory

After adding this extra information, one connection was found between a genetic component and a brain component. The brain component consisted of the inferior frontal gyrus which is part of the frontal lobe (see Figure 1). The left part of this gyrus is important for the understanding of language and is called Broca's area. The inferior frontal gyrus did not show a difference between patients with ADHD and healthy controls, but it did seem to influence working memory. Working memory is important for the short-term storage of information that can be used for decision making and behavior, and is one of the executive functions that is necessary for controlling our behavior. People with ADHD are known for having difficulties with the executive functions. They do not only have a problem with working memory, but also with other functions such as, attention, inhibition and cognitive flexibility. We did not find a link between the brain region and any of the other executive functions, nor with the normal symptoms of ADHD (inattention and hyperactivity).

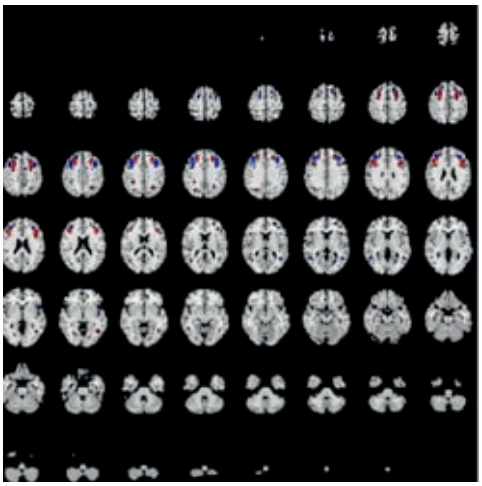


Figure 1. A spatial map of the brain component that includes the inferior frontal gyrus. The inferior frontal gyrus is indicated by the blue and red color.

But what do the genes do?

In the genetic component connected to this brain region, three genes were found that work in the brain (see Figure 2). The first one is CDH8, which works through calcium. CDH8 is involved in the formation of axons which form the connections between different brain regions. The second gene is ULK4, which regulates the neural stem cell pool. Neuronal stem cells develop into normal brain cells and help to make and maintain a well-functioning brain. This gene has also been found to be involved in the release of the neurotransmitter GABA. By releasing this neurotransmitter, the function of other neurons/connections in the brain can be slowed down. This corrects the overproduction of other neurotransmitters. The last gene is CPLX1. This gene contributes to the release of all neurotransmitters in the brain, which is necessary for the transductions of signals in the brain.

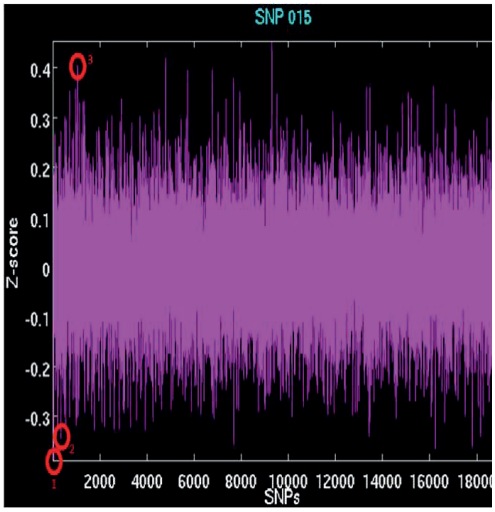


Figure 2. An overview of the genetic component. The highest peaks to either positive of negative indicates the genes in the component. The peak corresponds to a number and that number corresponds to a specific SNP. Peak indicated by red circle number 1 is CDH8, peak with circle number 2 is ULK4, and peak with circle number 3 is CPLX1.

What path can we take now?

We found genes involved in making connections between brain regions, and that the (inferior) frontal region is related to working memory. The combination of these support the idea that there is a connection between the frontal region and the cerebellum that is relevant to ADHD. The cerebellum is involved in the regulation and inhibition of movement, which can contribute to hyperactivity symptoms. The cerebellum is also involved in the other major symptom of ADHD, attention. Unfortunately, we were not able to find the cerebellum related to ADHD in this research.
The identified link between genetics and brain regions with working memory and executive functioning seems promising. Working memory should be explored more in combination with brain imaging, but also genetics. SNPs involved in cognition (executive functioning) have already been identified and so a possible future strategy is to include these SNPs as a reference to the parallel independent component analysis. This might reveal more clear results.

Special thanks to
In Nijmegen I want to thank Dr. Alejandro Arias Vasquez for teaching me the basics about genetics and programming, and Iris Duif (PhD student) for teaching me about MRI and other forms of brain imaging. In Albuquerque, I would like to thank Dr. Jean Liu for teaching me everything I needed to know to perform my research at the Mind Research Network in Albuquerque. I would also like to thank Kuaikuai Duan (PhD student) for bearing with me when I came to her with question number 1001. I won't forget the other members of our team that helped me in this field of mathematics and programming. And of course Prof. Vince Calhoun who opened up his lab for me and welcomed me into his team. And last but not least I want to thank Ryanne Offenberg for doing this program together with me in the Netherlands.

"The Canadian people welcomed me with arms wide open and invited me to lab activities, parties and even camping in the Rocky Mountains. Thanks to them I enjoyed every second of my time in Edmonton."

David Lamers

(Boxmeer, 1997)
Bachelor of Medicine: 2015-2018

Honours internship:	Department of Physiology, Radboudumc; Department of Physiology, University of Alberta, Edmonton, Canada
Supervisors:	Dr. Jenny van der Wijst, Prof. Dr. René Bindels; Dr. R. Todd Alexander

As expected, during the Bachelor of Medicine I learned a lot about the current knowledge of physiology and diseases, but actually that is just the conclusion of the story. I wanted to read the full book instead. The Honours Programme gave me the opportunity to learn more with regard to research.

I started the programme at the Department of Physiology in Nijmegen where I was introduced to renal physiology and basic laboratory techniques. The project in Nijmegen and the internship abroad were both focused on calcium handling in the kidney. Preparing for my stay in Edmonton was part of the internship in Nijmegen as well. Even though it was difficult to find a place to stay, everything worked out and I was fully prepared for the internship abroad. The Canadian people welcomed me with arms wide open and invited me to lab activities, parties and even camping in the Rocky Mountains. Thanks to them I enjoyed every second of my time in Edmonton. I learned to be independent, improved my English and made memories and friends for life.

Although the programme offered very interesting and instructive courses, I found it challenging to combine this with the requirements of the Bachelor degree every now and then. The thought 'I would have had a lot of free time if I didn't apply for the programme at all' crossed my mind several times. But every time I reminded myself of the things I had learned and the awesome internship that was to come, and I realized that it was definitely worth the effort. I am very grateful for this unique experience, for the skills I have learned, for the amazing people I have met and of course for getting to know the full story instead of just the last page of the book.

Dissolving Kidney Stone Formation: Calcium Handling in the Kidney

David Lamers

In Canada, one out of ten people will develop a kidney stone at a point in their life. In addition, inhabitants of other western countries have a 10-15% life time risk of developing a stone. This makes kidney stone disease very common and it has an increasing incidence across the world. Detecting and possibly treating the risk factors at an early stage can prevent the formation of kidney stones.

Calcium ions

One of the most important risk factors for the development of kidney stones is the amount of calcium ions excreted via the urine. If too much calcium is excreted, doctors will classify this as hypercalciuria. In some cases, a specific cause of hypercalciuria can be found which results in an adequate therapy. However, in most cases, no specific cause can be identified and therefore these people are diagnosed with idiopathic hypercalciuria. This is harder to detect and treat and may result in the development of kidney stones.

Claudin-14

The current study focused on claudin-14: a protein involved in calcium transport. Claudin-14 is part of the claudin family which consists of multiple variants of proteins, all of them with different characteristics with regard to ion transport. Claudins are present in the space between the cells of the kidney and form barriers or pores to respectively prevent or facilitate transport. In this study, claudin-14 is the protein of interest because it might contribute to the development of idiopathic hypercalciuria. Claudin-14 is present in a specific location in the kidney (the thick ascending limb of Henle's loop). It is known that claudin-14 interacts with two other claudins, namely claudin-16 and claudin-19. Claudin-16 and claudin-19 form a pore which allows calcium ions to flow back from the urine to the blood. However, when claudin-14 is present, this pore is blocked and calcium cannot go back to the blood. In other words, if there are too many claudin-14 proteins in the kidney, more calcium pores are blocked causing a higher urinary calcium excretion, which can result in hypercalciuria. Understanding how the expression of claudin-14 is regulated can help us find a specific target for treatment and therefore prevent the development of kidney stones.

Calcium sensing receptor

The rate of claudin-14 production (also called the expression of caludin-14) is regulated by the calcium sensing receptor (CaSR). It makes sure that a healthy calcium concentration is maintained: if the calcium concentration in the blood is too high, the CaSR ensures that more claudin-14 is present in order to prevent calcium going back to the blood, and stimulate the excretion of calcium via the urine. However, what we don't know is how the CaSR increases the expression of claudin-14, and which proteins

are involved in this mechanism. Because the CaSR is present in a large variety of tissue types, it has been widely investigated before. These studies described three pathways that are present downstream of the CaSR, illustrated in figure 1. All three start with different G-proteins directly downstream of the CaSR: Gi/o, Gq/11 and G12/13. The Gi/o pathway is the one on the left, which includes cyclic AMP (cAMP), the Gq/11 pathway is the one in the middle and includes protein kinase C (PKC), and last the G12/13 pathway with the Rho kinase family of which Cdc42 will be used in further experiments.

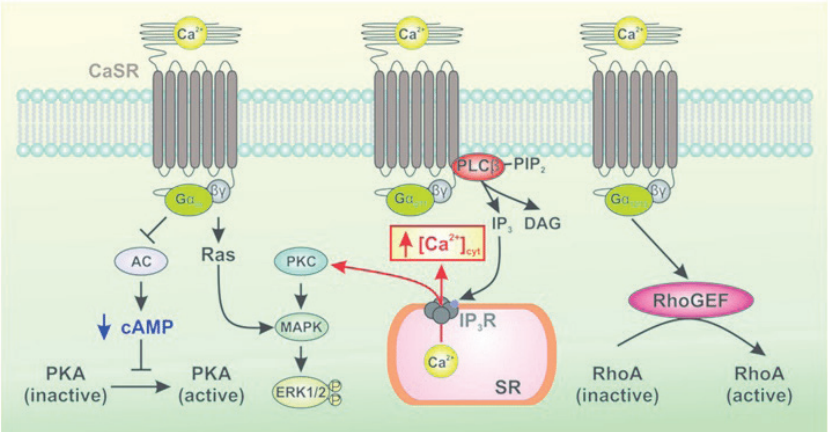


Figure 1. The three described pathways downstream of the CaSR

Cell culture model and experiments

To test if one of these pathways is involved in claudin-14 expression, human kidney cells were used. These cells were genetically modified with DNA constructs to make them express the proteins of interest, i.e. a promotor of the claudin-14 gene and the CaSR. The cells needed 24 hours to produce these proteins. After this day, the specific cells were treated with drugs to test which of the described pathways are involved in claudin-14 expression downstream of the CaSR. As mentioned before, three pathways were tested in separate experiments after the cell culture model was validated. To do so, Cinacalcet (an activator of the CaSR), Forskolin (results in an increase of cAMP), Staurosporine (a blocker of PKC) and a Cdc42 DNA construct were used to respectively validate the model, test the Gi/o, Gq/11, and G12/13 pathways. Finally, a luciferase assay was performed. This technique measures the emitted light caused by products made in the cells due to the genetic modification. The intensity of light is a measure to calculate the amount of claudin-14 expression.

Effects of the drugs and Cdc42 construct

As mentioned before, the experiments started with validating the cell model by treating the modified cells with Cinacalcet. When this drug was added to the cells, more light was emitted, which means that activation of the CaSR causes an increase in the expression of claudin-14. This is also exactly what was described in the literature, and so it can be concluded that the cell culture model is useful for these experiments.

After validation, all the other experiments contained four different conditions: one negative control, one where cells were treated with Cinacalcet, one with another drug of interest and one with a combination of Cinacalcet and the drug of interest.

The second experiment was focused on testing the Gi/o pathway for which Forskolin was the drug of interest. These results showed that the light intensity in the Cinacalcet group and Forskolin group was approximately equal, but the effect of the combination of Cinacalcet and Forskolin was significantly higher compared to either drug alone.

This finding was also seen in the third set of experiments. Staurosporine was used in these experiments to investigate the Gq/11 pathway. Comparable to the Forskolin set, light intensity of Cinacalcet and Staurosporine alone was significantly lower compared to the combination of both drugs.

Lastly, Cdc42 DNA constructs were used to test involvement of the G12/13 pathway. Three different constructs were used: a construct that does not contain the coding part for the protein, a functional construct that resembles the one found in the human body, and a mutated construct that is not functional. Interestingly, the effect of Cinacalcet seemed slightly decreased when Cdc42 was present, which might suggest that Cdc42 has an effect on the claudin-14 expression.

Conclusion

As mentioned before, the combination of Cinacalcet and Forskolin, and the combination of Cinacalcet and Staurosporine gave a higher light intensity compared to the cells treated with these drugs on their own. This means that the effect of Forskolin and Staurosporine is additive to the effect of Cinacalcet. Thus, the drugs do not address the same pathway and therefore can be concluded that the Gi/o and Gq/11 pathway are not involved in claudin-14 expression as a result of CaSR activation. However, the Cdc42 experiments showed different results. The decrease of light intensity in cells treated with Cinacalcet and with the presence of Cdc42 implies that the effect of Cdc42 is not additive to the effect of Cinacalcet. This would mean that both Cinacalcet and Cdc42 act on the same pathway and thus that the G12/13 pathway is involved in claudin-14 expression. However, because there was not enough time to repeat these experiments, no valid conclusions are available yet with regard to G12/13. Nevertheless, it is likely that the G12/13 pathway is the pathway involved since the other two pathways are excluded.

It is recommended to repeat the Cdc42 experiments in future research in order to increase the validity of the results. Once the involvement of the G12/13 pathway has been confirmed, research can focus on ways to intervene in this pathway if claudin-14 is overexpressed and improve the treatment of idiopathic hypercalciuria and the prevention of the development of kidney stones.

Special thanks to
I would like to thank Dr. Jenny van der Wijst and Prof. Dr. René Bindels for giving me the opportunity to prepare myself in the laboratory at the Department of Physiology at the Radboudumc. Second, I would like to thank Dr. R. Todd Alexander for his hospitality and guidance during my internship at the Department of Physiology at the University of Alberta. Finally, I thank the Alexander lab members and all the other people I have met in Canada for helping me whenever I needed help and for making my internship a true pleasure.

Julian Lieverse

(Utrecht, 1995)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Geriatrics, Radboudumc; Department of Physiotherapy, Trinity Centre for Health Sciences, St. James's Hospital, Dublin, Ireland
Supervisors:	Sanne Gijzel, Jana Thomas, Prof. Dr. Marcel Olde Rikkert; Kate Devenney, Prof. Dr. Brian Lawlor

Someone asked me what I liked most about my internship in Dublin. Was it my supervisors? The interesting research topic? The patients involved in the study? The students I met from around the world? The city and beautiful country itself? Receiving friends and family and give them a tour through Dublin? Maybe the pints of Guinness? Actually, it is impossible to decide what I most liked because there were so many activities and great people I met during my internship.

More than two years ago, a journey called "Honours Programme Medical Sciences" began. I started my internship on the department of Geriatrics. This first period was focused on the research theme resilience. I was directly involved in a study and my preference for geriatrics increased. I also had the opportunity to get to know the department well and do research.

In Dublin I switched to another research topic: exercise in relation to sleep problems in patients with cognitive complaints. Every week I had the opportunity to supervise exercise classes attended by these patients. Because of the humorous Irish people, this was definitely a pleasure. Although Dublin is less than 1000 kilometres away from the Netherlands, it is very different to Dutch cities. But it's very beautiful, as are other parts of Ireland such as the West Coast.

The Honours Programme Medical Sciences has given me the opportunity to meet with a lot of interesting people from around the world, to gain knowledge about a lot of different fields of research, and of course to do research myself. Because I had such an inspiring and instructive period on the Geriatric department, I will continue doing research on this topic.

Sleep problems in patients with cognitive decline: exercise!

Julian Lieverse

We all know how frustrating a sleepless night can be. Being awake for hours, rolling from one side to another and in the meantime seeing the hours passing by on the alarm clock. Nothing seems to work. Luckily for most of us, bad nights like those don't occur often and the following night is usually much better. Can you imagine how frustrating it must be if this happens almost every night? This is often the case in people with cognitive decline.

Cognitive decline

People with cognitive decline, also known as mild cognitive impairment (MCI), have problems in their brain function. MCI is defined as cognitive decline greater than expected for someone's education level and age, but which does not have a significant impact on activities of daily life. It is estimated that between 10 and 20% of people aged 65 years or older suffer from MCI. One of the most common symptoms seen in patients with MCI is memory decline. Other complaints include: trouble with planning, an increased risk of developing depression, and sleep disturbances. This last complaint is especially important, because between 14 and 59% of the people with MCI report sleep disturbances.

Different factors are associated with sleep quality in MCI patients. For example people with MCI who suffer more from depression also report more sleep problems. Furthermore, the severity of cognitive decline is a factor influencing sleep quality in MCI patients. People with greater cognitive decline report more sleep disturbances. It is found that sleep disturbances cause more cognitive decline, creating a downward spiral. It is therefore important to treat sleep disturbances in people who suffer from MCI.

Treating sleep problems

Different treatment options are available to improve sleep quality. It all begins with so-called 'sleep hygiene'. Sleep hygiene consists mainly of behavioural changes. For example not drinking coffee late in the evening, reducing the intensity of blue light just before you go to bed and not using your smartphone when you are in bed. However, sometimes sleep hygiene adjustments are not enough and other options are needed.

We all know pharmaceutical treatments. Taking sleep medication seems an easy option to get rid of sleep problems. But its efficacy is questionable and there are a number of side effects, for example an increased risk of falls. It is also possible that patients may develop a dependency, which leads to higher doses while the effect of the medication decreases. Especially important among people with MCI is the finding that sleep medication can lead to further cognitive decline. Because treatment of sleep problems with medication is not the best option, other options are needed.

Is exercise worth a try?

After a heavy workout in the gym, an intense match on a football field or a long distance run, you have probably noticed that you sleep well that night. It is therefore not surprising that it was hypothesized that exercise would improve sleep quality in MCI patients. To test this hypothesis, different studies have been done over the last few years. Those studies focused on different forms of exercise.

Roughly, exercise can be divided into 'aerobic exercise' and 'non-aerobic exercise'. Aerobic exercise is defined as exercise that increases heart rate with the aim of improving cardiovascular fitness. Examples are walking, swimming and cycling. Non-aerobic exercise consists of stretching, resistance training, muscle toning or maintaining balance. Intervention studies done with MCI patients in order to examine sleep quality have so far been limited in one or more ways. Such studies have only used aerobic exercise as an intervention, for example. No study has been done which examined and compared the effects of aerobic exercise and non-aerobic exercise on sleep. Previous studies are also often limited in the number of participants, the absence of a control group or the duration of the interventions.

A new study

In 2016 the 'NeuroExercise Study' started. The aim of the NeuroExercise Study was to examine the effects of aerobic and non-aerobic exercise in MCI patients. Therefore people aged 50 years or older with a clinical diagnosis of MCI were recruited in Dublin (Ireland) and Nijmegen (The Netherlands). They were randomised into one of the three study groups: an aerobic exercise group, a non-aerobic exercise group or a control group.

Before the groups started with their exercise intervention (at baseline), different questionnaires about depressive symptoms, cognition, physical activity and sleep quality were administered. The sleep questionnaire consisted of seven components of sleep, for example, how easy someone falls asleep and how many times they wake up during the night.

Exercise classes were performed three times per week over a period of six months. One exercise class consisted of one hour of the appropriate exercise, aerobic or non-aerobic. Participants in the control group were not instructed about exercise or asked to attend classes. After six months the same questionnaires used at baseline were used again. This made it possible to compare the baseline scores with the scores after six months exercising.

Effects of exercise on sleep

Our study shows that a six month aerobic exercise period is an effective method to improve sleep quality in people with MCI. Sleep scores in the aerobic exercise group were more improved compared to the group that did not exercise. They reported less difficulties falling asleep, less sleep disturbances and better daytime function. In contrast, non-aerobic exercise was not effective in improving sleep quality, as both the non-aerobic exercise group and the control group had the same sleep scores. Since sleep disturbances are often seen in people with MCI, a recommendation to improve sleep quality for this group is therefore aerobic exercising such as running, swimming or cycling. Despite our knowledge that aerobic exercise improves sleep quality, we don't know the exact mechanism which causes this effect. The improved sleep scores in the aerobic exercise group might not be a direct effect of exercise, but might rather be a result of

less depressive symptoms. Previous studies have shown that depressive symptoms can negatively influence sleep quality. Our study found less depressive symptoms in the aerobic exercise group compared to the control group after the exercise period. However, there was no difference between the non-aerobic exercise group and the control group. Therefore it is possible that aerobic exercise leads to less depressive symptoms and this, in turn, leads to improved sleep quality. Further research is needed to examine the effect of exercise on sleep quality and depression and the relationship between those two factors.

Acknowledgements
Fortunately, I didn't experience any sleepless nights during my internship thanks to the help of the following people. First of all, my supervisors in Ireland: Kate Devenney and Prof. Dr. Brian Lawlor. They were incredibly helpful throughout my internship and made me feel at home. Of course I also want to thank my Dutch supervisors for their motivational guidance of my internship: Sanne Gijzel, Jana Thomas and Prof. Dr. Marcel Olde Rikkert. They made me even more enthusiastic about geriatrics. Lastly, I want to thank the wonderful and humorous participants of the NeuroExercise Study in Dublin and Nijmegen. Without them this study would never have been possible.

Ryanne Offenberg

(Oss, 1997)
Bachelor of Medicine 2015 - 2018

Honours Internship:	Department of Human Genetics, Donders Institute for Brain, Cognition and Behaviour, Radboudumc; Department of Psychiatry, Psychosomatics and Psychotherapy, University Hospital Frankfurt am Main, Goethe University, Germany.
Supervisors:	Dr. Alejandro Arias Vásquez; Dr. Oliver Grimm

The honours programme was an opportunity I couldn't miss out on. I found myself missing the 'science' part in medicine and wanted more. Now I had the option to combine the two!

Visiting the different research institutes during the first year of the programme was very interesting. The different labs I visited, the different research topics I learnt about, and the different people I met, all excited me. I acquired a little bit of experience in a wide range of research topics: from learning how to handle a pipette to performing some basic statistical analyses in the software programme 'R'.
Additionally, I learned how to plan in a more flexible way and prioritize my time better. It could be challenging at times to combine the honours programme with my bachelor's degree. The different schedules could be far from ideal and sometimes my to do-list seemed never-ending. Looking back, I'm glad I pulled through.

To travel, work, and live abroad was an incredible experience. Germany might not sound all that exciting, and it is certainly not the same as going to New York, but my time there remains something I will never forget. Cycling across the busy streets in Frankfurt, living on my own for the first time, working in a completely different environment, and meeting very kind people from all over the world. I also found that devoting myself to research and diving deep into a specific subject is something I really enjoy doing. That is why I have made the decision to do a master's program in medical imaging, combing mathematics, physics, and programming with medicine.

In short, by participating in the honours programme I have increased my knowledge, developed new skills, and I have grown as a person.

Mental disorders and comorbidities: still a mystery...

Ryanne Offenberg

Mental disorders often co-exist with other conditions: comorbidities. When someone is suffering from a mental condition, he or she becomes more vulnerable to the development of other (mental) disorders. The impact of such a condition on someone's life can grow immensely due to this accumulation. Why is this happening and is there a way we can prevent these comorbidities from developing?

ADHD and comorbidity

Before specific methods to prevent comorbidity can be developed, we have to learn more about the pathological mechanisms that are responsible for the development of comorbidities in mental disorders. Right now, this knowledge is very limited. The CoCA-project (Comorbid Conditions of ADHD) is one of the research projects that is working to gain more insight into the complexity of comorbidity in mental disorders. As the name implies, this multicentre project works from the perspective of attention deficit hyperactivity disorder (ADHD). ADHD is a disorder that starts early in life, meaning it precedes most comorbidities. Also, more than 80% of adult ADHD patients suffer from comorbidities, such as mood and anxiety disorders, substance use disorder, or obesity. This means that the underlying pathological mechanisms of ADHD can help us understand the development of comorbidities.

Functional MRI

For my internship, I worked in a subgroup of the CoCA-project that was specialized in functional magnetic resonance imaging (fMRI) research. fMRI is a method to study brain activity using an MRI-scanner. This is a scanner that can produce images of objects (including body tissues) based on differences in magnetic properties. With different settings, you can display a variety of properties of an object. 'Functional' in fMRI refers to MRI-scanner settings that allow you to visualize brain activity. In figure 1, you can see an example of a visual representation of brain activity.
We used a specific method that captures blood oxygen level dependent (BOLD) signals in the brain. The idea is that when a specific brain area is activated, it will need more oxygen. This means that blood with high concentrations of oxygen (oxygenated blood) will go to that specific brain area, followed by a change in the ratio between oxygenated blood and blood with low concentrations of oxygen (deoxygenated blood). Because oxygenated and deoxygenated blood have different magnetic properties, they affect the magnetic field differently, creating a BOLD signal.

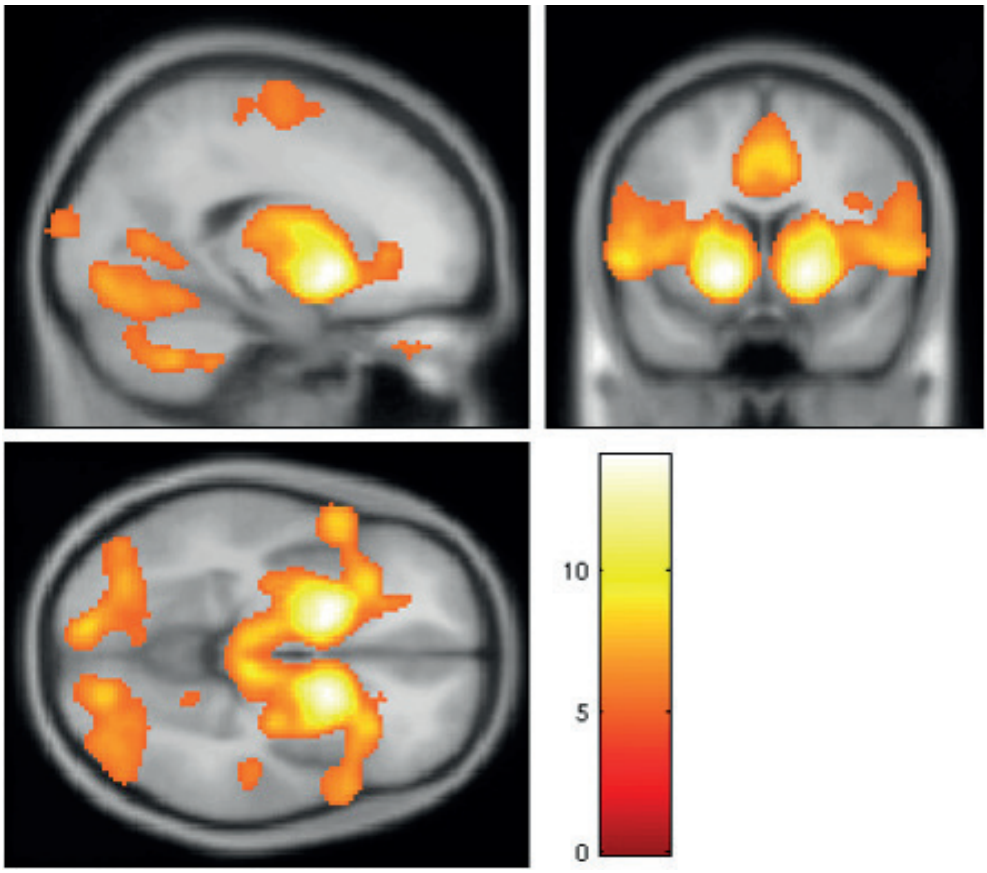


Figure 1. Overview of an average BOLD signal in the brain. The brain regions with the strongest activity, are indicated with the yellow/white signal.

Dopamine and reward

The purpose of our fMRI study, was to analyse the role of dopamine in ADHD and comorbidities. Dopamine is a so-called neurotransmitter: a chemical that is released by a cell of the central nervous system to communicate with another cell. It plays an important role in reward processes and ADHD is associated with decreased dopamine activity.

A complete scanning session consisted of a structural scan and a few functional scans. Healthy participants were scanned three times at different time points. Each time, a different drug was administered: a drug stimulating the effect of dopamine, a drug blocking the effect of dopamine, or a placebo. This was to compare their performance when affected by the different drugs.
My job was to assist some of the scanning sessions, process the data we gathered, perform the first statistical analyses and interpret them, as well as performing data quality checks. Data processing consisted of correcting the functional images for movement, aligning them onto anatomical images, and other processes that were needed before all the images could be compared to each other. Unfortunately I can't share any specific outcomes of my analyses. Simply, because the study is still ongoing and my data was not complete. For the

honours programme, I did conduct my own little study concerning a specific magnetic field correction. In the end, this correction was not implemented as part of the general data processing pipeline of the CoCA study, because it didn't improve the data quality in the reward-related brain areas.

In conclusion

The CoCA-project is still running on full speed and people are working hard to solve the mystery surrounding comorbidities. Perhaps in the near future, we will know more and can develop new ways to help people. We may even be able to prevent the development of comorbidities.

Special thanks to
Dr. Oliver Grimm, my supervisor at the university hospital in Frankfurt am Main. Dr. Mortitz de Greck, for teaching me how to read (and write!) perl scripts. Prof. Dr. Ralf Deichmann, for teaching me about MR physics. Charlette Diercks and Leona Fey (neuroscience master students) for being wonderful partners to work with. Dr. Alejandro Vasquez, my supervisor in Nijmegen. Iris Duif (PhD student), for teaching me about the basics of neuroimaging. Claire Koeyvoets for working with me during our time in the Netherlands.

Suzanne van Ooij

(Nijmegen, 1998)
Bachelor of Medicine 2015-2018

Honours internship:	Donders Institute for Brain, Cognition and Behaviour, Radboudumc; Monash Institute of Cognitive and Clinical Psychology, Melbourne, Australia
Supervisors:	Martin Dresler; Clare Anderson

In the beginning, the biggest challenge was to not panic looking at the conflicting schedules of the Honours programme and my Bachelor's degree. In the end, the biggest challenge was not to panic having to present my 3-month research project to a group of professionals. Surprisingly, I found the latter to be much easier.

During these last two years, we had the incredible opportunity to learn about all kinds of research. From trying to sound like a professional English-speaking researcher and listening to accomplished researchers every Thursday afternoon to flying off to the other side of the world wondering how I'm ever going to complete my own study: the last two years had it all.

I had to sacrifice the exceptionally hot Dutch summer for the cold and rainy Melbourne winter, but that did not take away from the amazing experience I had. Firstly, I learned that apparently not all Australians go surfing every weekend and have mid-length hair in beachy waves. However, it is true that they are incredibly kind and welcoming to strangers and newcomers, so I was very fortunate. Having lunch and chitchatting with fellow Honours students and lab members definitely made the long days behind a computer easier to get through. Learning about Aussie culture (and Asian culture, thanks to my housemates) was also great fun - except for tasting Vegemite - and made me feel at home 16,500 km from home.

All in all, the Honours Programme has been a wonderful (and sometimes stressful) experience. Discovering parts of the hospital and the world I didn't think I ever would, I truly learned a great deal about doing your own research. I'm very grateful that I got the opportunity to do all these amazing things.

Reading scientific tealeaves: looking at REM sleep to prevent mood disorders

Suzanne van Ooij

You may not notice it, but while you are finally getting your well-deserved hours of sleep after a long day, a lot of interesting things are happening in your brain. Consolidating new memories, processing your emotions, boosting your immune system, getting rid of the waste products from your cells and much more. One of the most elusive phenomena is REM (Rapid Eye Movement) sleep. While you may have never heard of the term, you are familiar with its effect on you: realistic dreams. My research focused on finding out what the density of this REM sleep can tell us about mood in patients with subjective memory complaints.

What are subjective memory complaints?

When people experience problems with their memory, but do not show deficits on objective memory testing measures, they have subjective memory complaints (SMC). These problems are very frequent among healthy individuals: 17% to 56% experience SMC. This self-reported memory decline increases the risk of dementia in older adults twofold and is seen as a preceding stage to mild cognitive impairment, where you can see additional abnormalities in cognitive tests. Next to spending minutes trying to recollect what appointments they made for the day, people with SMC are more often depressed and anxious in comparison to healthy individuals.

Older adults with SMC are thus more likely to experience low mood. Self-report mood scales are biased towards external instead of internal states because mood is easily influenced by many external factors in daily life. Just think about how your mood over the day is influenced by small irritations such as traffic jams, an argument or bad weather. An objective measure of mood is missing, but would be beneficial for objectifying and tracking mood in this group with SMC.

What is REM density and how do you study it?

Throughout the night, you complete multiple cycles of successive sleep stages. REM sleep is the last stage in this repeating cycle. Not surprisingly, REM sleep is characterised by rapid eye movements, lasting less than 500 milliseconds. REM density describes the frequency of these rapid eye movements during REM sleep, usually expressed as number of REMs per minute of REM sleep. These REMs can be measured by using polysomnography (PSG), which uses electrodes to measure brain activity, eye movements, muscle activity and heart rhythm. The eye movements can be seen on the product of PSG as sharp peaks. By simply counting (or letting a programme calculate) the amount of these movements and dividing it by the time in REM, you get the REM density.

Why REM density and SMC?

Previous research has shown that REM density is increased in patients with mood disorders such as depression and bipolar disorder. Most studies found this effect for the first REM cycle only, in other words the first time you go through REM sleep in the night. Interestingly, one study showed that REM density is even predictive for the onset of mood disorders and is heightened in healthy relatives of depressed patients. This means it is possibly a marker for depression and other psychiatric disorders. This kind of marker is called an endophenotype: a heritable, measurable, state independent, biological marker for an illness. It is mostly used to better understand the biological processes underlying a disease.

REM density is also related to memory performance: people remember sad stories better when their REM density is higher. The Sleep to Forget Sleep to Remember (SFSR) hypothesis might explain why that is. This SFSR hypothesis suggests that during REM, cholinergic activity is dominant. This means that the neurotransmitter acetylcholine is used at higher levels than other neurotransmitters. Neurotransmitters can be seen as messengers in the brain that contain the information on what to do. Acetylcholine is one of these messengers. The SFSR hypothesis poses that the cholinergic activity enables reprocessing of emotional memories, which is why the memory core of these emotional memories is strengthened.

Since REM density is related to mood disorders, and SMC is related to poor mood, the research question was whether REM density is a biomarker for poor mood outcomes in healthy older people with subjective memory complaints.

How did we do this?

In 29 older adults between 60 and 80 years old with SMC, we measured two consecutive nights of their sleep with PSG. The first night worked as an adaptation night to get used to sleeping in an unknown environment. We calculated the REM density using data from the second night. Next to the sleep recordings, participants filled out multiple questionnaires on depressive symptoms, anxiety, stress and general psychological health. Because the participants all had subjective memory complaints, we also included two questionnaires to quantify these memory complaints.

What did we find?

For almost all REM cycles, men had higher REM densities than women. At the end of the night, REM density also differed with age: the older participants were, the higher their REM density even though age had no relation with mood. As for the quantity of memory complaints, people with more memory problems also reported higher stress and depression scores but there was no relation with REM density. Surprisingly, we found the REM density of the second cycle to be related with anxiety scores and especially levels of stress. To a smaller extent, first cycle REM density was associated with depression scores.

How can we explain these results?

As previously mentioned, the relation between REM density and mood can be explained by the neurobiological mechanisms of REM sleep. Very basal parts of the brain modulate brain activity through cholinergic activity, which initiates and regulates REM sleep as a sort of manager. A previous study showed that cholinergic sensitivity is higher in patients

with mood disorders, which in turn could explain the differences in REM sleep: a higher sensitivity for cholinergic activity could mean extra activity in REM.

To our surprise, this is the first study that found the second cycle of REM as the strongest correlate of mood instead of the first cycle. It is not completely clear why that is; one possibility could lay in the influence of circadian rhythm of REM density, which was not taken into account. The circadian rhythm is the internal daily rhythm of your body. For example, it enables you to wake and get up at a certain time in the morning – or not.

What does this mean?

Based on these results, second cycle REM density shows promising results as a biomarker for poor mood in a healthy population of older adults with subjective memory complaints. We achieved these results even though we had a restriction in mood scores, because we excluded depressed individuals. Second cycle REM density could therefore be useful as an objective measure of mood in healthy people with SMC, and possibly even as a predictor of worsening mood. Since depression and anxiety are common problems among people with SMC and this leads to decreased quality of life, predicting these issues could be essential for early intervention.

Acknowledgements
Without the help of my UMCN supervisors, Dr. Martin Dresler and Leonore Bovy, and my supervisors from Monash, Clare Anderson and Jessy Manousakis, I would not have been able to conduct this study. For that, I am very grateful. I would also like to thank Frederik Weber for letting me use SpiSOP for calculating REM density and helping me getting it to work.

Boyd van Reijmersdal

(Nijmegen, 1996)
Bachelor of Biomedical Sciences
2015-2018

Honours Internship:	Department of Cognitive Neuroscience, Radboudumc; Department of Pharmacological and Biomolecular Sciences, University of Milan, Milan, Italy
Supervisors:	Michel Verheij, Judith Homberg; Lucia Caffino, Fabio Fumagalli

These two years of the Honours Programme really made my bachelor's degree. While everybody was fighting their schedules due to the messy new curriculum I didn't care that much because I had the opportunity to learn things twice as fast with hands-on, personal projects within the programme. These included a trip to Oxford, guided tours, lab work with researchers and so on. Besides these the programme brought together a group of enthusiastic motivated people with whom I shared two very pleasant years.

The first year was very useful with learning some English skills and getting in touch with researchers from many different fields. This helped me a lot with choosing the direction of my further career in research. Eventually I found myself attracted to the wondrous world of the brain. I went searching for a topic that really interested me, without considering the destination of the projects. It was a welcome surprise that the most interesting project had a collaboration in my favorite country: Italy!

And off I went, 5 months in the city of good food, football, and fashion; Milan. I learned a lot during the many hours I spend in the laboratory. Obviously about the topic of how addictive behavior affects our brains, but also about eating pasta, drinking coffee, speaking with gestures, and the Mediterranean attitude.
However, most of the memories have been made in the evenings and weekends, outside of the lab, where I made amazing friendships. With these people from all over Italy, Europe, and the world I explored the beauty Milan and Italy have to offer, from hiking in the Alps to drinking wine in Tuscany.

Now, I have started my master's degree in neuroscience and while doing so I try to find moments to make trips across Europe where I have many couches to crash on!

Exploring the addiction vulnerable brain

Boyd van Reijmersdal

Substance abuse disorder is a condition that is often thought to be the patient's own fault. Nobody ever accidentally injected heroin or sniffed cocaine, and people have the choice not to take it, right? But what if I tell you that everything we experience changes our brain. These changes can eventually lead to compulsive drug abuse behavior. And some brains are more vulnerable to these changes than others. With our research we tried to study some of these differences in vulnerability to addiction by using a rat model of cocaine addiction.

What is drug addiction?

Drugs are experienced as pleasant because of their stimulating effect on our brain's reward system. Recreational drug use (impulsivity) can develop into compulsive drug use (compulsivity) by repetitively going through a cycle of these three stages: binge/intoxication, withdrawal/negative affect, and drug seeking/craving. Every time somebody goes through such a cycle their reward system is being activated and bombs of dopamine, serotonin and other neurotransmitters explode in the brain causing a cloud of happiness/relaxation/focus. However, every time this takes place, the brain changes a little bit, making the brain less responsive to the drug and to the brain's own neurotransmitters. The result is that without the drug, the brain is incapable of maintaining a 'normal happy state'. This is the basis of withdrawal which eventually drives the person to crave for the drug again. Eventually a drug addict is diagnosed by three clear criteria: increased drug intake over time, continuing to use the drug despite the negative effects, and becoming physically and/or psychologically dependent on the drug.

Some are more vulnerable than others

One in ten people has a genetic variability that makes them more vulnerable to anxiety, depression, and substance abuse. This genetic variability is situated in the serotonin reuptake transporter (SERT) which can be seen as a vacuum cleaner for the 'well-being and happiness' neurotransmitter serotonin. These people are thought to have less control over their mental state due to this difference. This, however, is not always negative. You might have heard about a 'high-sensitivity personality' which seems to be strongly related to this genetic variability. People with these characteristics are also more sensitive for their environment and more creative individuals.

In this study we used two types of rats which were given cocaine, the second most abused drug in Europe. The first type are rats that have the gene coding for SERT knocked out. (SERT KO rats) These SERT KO rats show the same high-sensitivity personality characteristics as humans and also show increased vulnerability to anxiety, depression-like behavior, and addiction. We compared the reaction of these rats to cocaine to reactions of the second group: normal wildtype rats (WT rats).

Pressing buttons for cocaine

We aimed to find answers to the question of why this genetic variance makes some more vulnerable to addictive behavior than others, by comparing SERT KO rats to WT rats. Rats received a catheter in a big vein in their necks through which a cocaine solution could reach the brain in a fast way. We used a cage in where rats received a shot of cocaine every time they pressed the correct lever, as illustrated in figure 1. During the first ten training sessions rats spent one hour per day in the cage in order to habituate to the procedure. After this, rats were assigned to either 15 sessions of one hour (short access/ShA) or six hours (long access/LgA) in the box as a model for impulsive and compulsive intake. Next to these two groups we used a group of naïve rats that were only implanted with the catheter without being exposed to cocaine. So in the end we have six groups: naïve, ShA, and LgA treatment groups for both KO and WT rats.

As data came in it became clear quite quickly that the general hypotheses were supported. The rats showed increased intake of cocaine over time and in SERT KO rats this escalation happens more early than in WT rats. Graphs for the LgA groups are shown in figure 1 and are analogue to the ones for the ShA groups.

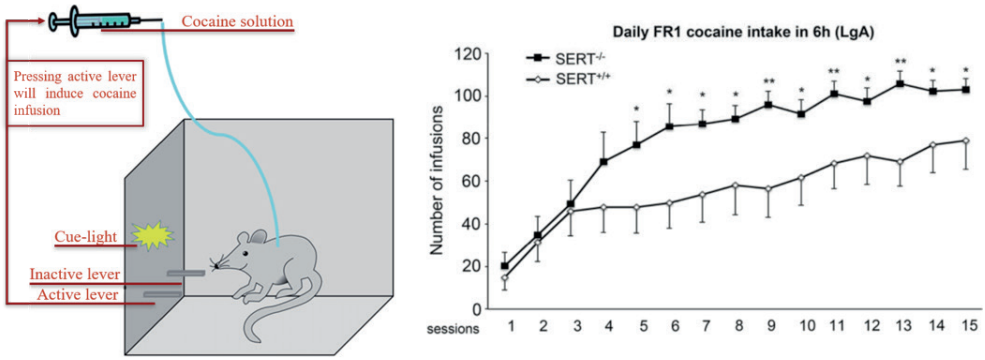
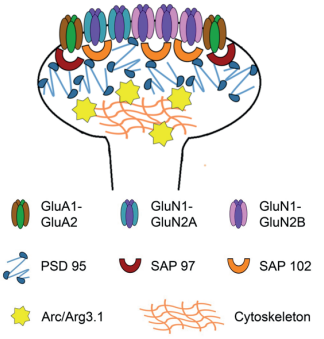


Figure 1. Self-administration experiments. Left; Self-administration of cocaine is achieved by providing the rat with an intravenous catheter and placing it in a skinner box where the rat receives an infusion every time they press a lever. Right; The cocaine self-administration paradigm is able to induce increased drug intake over daily sessions of 6 hours. SERT knockout rats show a significant increased cocaine intake as compared to their wildtype counterparts. SERT-/-=SERT KO, SERT+/+=WT

Changes in crucial brain regions

After studying their behavior, the rat's brains were removed for further analyses. The interesting brain regions were taken out with a hollow needle for further analysis. The region we chose to study first is called the infralimbic region of the prefrontal cortex (IL). This region is situated in the frontal region of the brain which is known for rationale and cognitive control of the more inner regions responsible for anxiety, sexuality, and also drug craving. It is like the angel telling the devil not to take the drug. In this IL region, we measured protein levels of the glutamatergic synapse (figure 2) which correlate with the sensitivity of the region to be activated. So, in a simplified way we could say that increased presence of these proteins implicates a more sensitive brake on drug craving, and that decreased levels are like a dysfunctional brake on drug craving.

Some of the things we found could be easily explained and some, luckily, we could not, as science becomes exciting when you get unexpected results. As shown in figure 2, there was a tendency of the markers going down in the LgA group of the normal/WT rats. A similar decrease could be observed in the baseline/naïve group of the high-sensitive/KO rats. These decreases could be described by the theory of a decreased sensitivity of the brake that should control drug intake. However, a pronounced and difficult to explain increase was found in the ShA group of the high-sensitive/KO rats. This could be caused by a compensatory mechanism in where the brain tries to fix the brake and rescue the organism from the harmful effects of the drug. Or, since the ShA group intends to model impulsive/recreational use, this might be explained as an increased brain reaction to relatively low exposure of the drug, serving as a possible solution to the question why these high-sensitive rats (and humans) are more vulnerable to addiction.



	GluA1/ GluA2	GluN1/ GluN2A	GluN1/ GluN2B	PSD95	SAP97	Arc
WT naïve	-	-	-	-	-	-
WT ShA	-	-	-	-	-	-
WT LgA	↓	↓	↓	↓	-	↑
KO naïve	↓	↓	-	-	-	-
KO ShA	↑↑	↑↑	↑↑	↑↑	↑↑	↑↑
KO LgA	-	-	↓	-	-	↓

Figure 2. Summary of synaptic changes. Left; A standard glutamatergic synapse consists of 3 main subtypes of receptors build on a fundament of several scaffolding proteins. Right; Changes in quantity of proteins present in every group as compared to the WT naïve (control) group, measured with western blotting. ShA=Short Access, LgA=Long Access

Good research never ends

Cocaine exposure changes the brain of a high-sensitive personality in a different way than their controls. Explaining the observed differences remains difficult, especially for the increased markers in the ShA KO group. But, within a short time, other regions of the same network will be investigated. This will hopefully shine a light on the remaining questions and should eventually lead to a more personalized approach for patients.

I would like to end with this concluding remark: our study contributes to the knowledge that brains can change without somebody to blame, leading to loss of control resulting in diseases like aggression, depression, and addiction, like a virus taking over a computer.

Acknowledgements
I would like to thank my supervisor in the molecular laboratory in Milan, Dr. Lucia Caffino, and all other members of the laboratory for their wonderful assistance and kindness. I wish to thank Dr. Michel Verheij and Stephanie Seeger for their guidance during the behavioral experiments in Nijmegen. Also, I would like to offer special thanks to Prof. Fabio Fumagalli and Prof. Judith Homberg for providing me the opportunity to do my BSc internship with behavioral experiments in Nijmegen and a substantial molecular part in Milan.

Charlotte Roosendaal

(Baarn, 1996)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Physiology, Radboudumc, Nijmegen; Department of Internal Medicine, Nephrology, Yale University, New Haven, USA
Supervisors:	Eric Verschuren M.Sc., Anna-Rachel Gallagher PhD
Principal Investigators:	Prof. Dr. Joost Hoenderop, Prof. Dr. Stefan Somlo.

September 5, 2016 was the day this journey started. We signed an agreement, devoting part of the next two years to the Honours Programme. After the English course, which definitely paid off I discovered, we were introduced to the practical side of research and the many possibilities it has to offer. From all these possibilities we had to choose our own internship. What topic? Which country? How far away from home do I want to go?

Making choices has never been my best quality and many topics presented interested me. However, when I heard we could also arrange an internship ourselves, I was immediately up to the challenge. Together with Dr. Prof. Joost Hoenderop I realized the amazing opportunity to do my internship at Yale University, under supervision of one of the major intellectual forces in renal physiology. In preparation to my stay there, I spent eight months in the department of Physiology at the RIMLS, where I was taught the basics of research and got acquainted with Polycystic Kidney Disease, until finally the time arrived which I had been looking forward to for so long: going to the USA.

Today, two years after the start of the program, I am writing this from my 'home away from home' in New Haven. I am nearing the end of my internship and can proudly say I have grown into a confident student researcher. Not only did I learn a huge variety of laboratory techniques, how to interpret the results and plan what to do next; I learned the ins and outs of Polycystic Kidney Disease, the importance of research and how devoted my colleagues are to their jobs. I have met wonderful people, made some really good friends and have seen and experienced so much of the United States and Canada. It has been an amazing experience, which will never be taken away from me.

Unravelling the role of Fibrocystin in Planar Cell Polarity

Charlotte Roosendaal

Can you imagine a life in which your survival is dependent on a machine? A life revolving around doctor's appointments, schedules and diets? Unfortunately, this is the daily truth for many Polycystic Kidney Disease (PKD) patients. PKD is a common genetic disorder that is characterized by the formation of numerous fluid-filled cysts in the kidneys and liver, causing the kidneys to expand from the size of a human fist to sometimes as large as a football. The disease exists in both a dominant (ADPKD) and recessive (ARPKD) form. ADPKD is caused by a mutation in PKD1 (85%), PKD2 (14%) or GANAB (1%), whereas ARPKD is caused by a mutation in a single gene, PKHD1. With an incidence of 1 in 1000 people worldwide, ADPKD is by far the most common inheritable kidney disease. However, currently, no cure exists for this disease and treatment focuses on reducing symptoms, with the ultimate treatment being a kidney transplant. This leaves a lot of room for improvement – and this is where research comes in.

Kidneys in health and disease

The kidneys play an important role in maintaining homeostasis in the body. Most people know the kidneys are responsible for urine production. However, before urine is actually created, an elaborate process is going on, regulating blood pressure, acid-base balance, electrolyte concentrations and the volume of fluid in our body. In order to be able to do this, the kidney is made up of approximately 1 million nephrons, each of which consists of a filter unit (the glomerulus) and a long tube (the tubule) (Fig. 1A) which together eliminate waste and excess fluid from our blood. The tubule is lined by a single layer of cells called epithelial cells, which mediate the exchange of fluid and ions between the fluid in the tubules and the interstitial fluid. However, in PKD patients, the lining of the tubule is not smooth. In ADPKD, multiple areas of the tubule can balloon out, which soon pinch off to form cysts throughout the entire kidney (Fig. 1B). In contrast, the formation of cysts in ARPKD differs both in location and appearance, as cysts are more fusiform-like and confined to the collecting duct (Fig. 1C). As the number and size of the cysts increases, they start interfering with normal kidney function, resulting in symptoms such as high blood pressure, blood in the urine and urinating excessively often. Though the presentation of cysts may differ between ADPKD and ARPKD, the exact mechanism of cyst formation remains unknown in both cases. However, a signal transduction pathway called Wnt signaling has been implicated with cyst formation. Two major distinctive branches of Wnt signaling exist, the canonical (β-catenin dependent) pathway and the noncanonical (β-catenin independent)/Planar Cell Polarity pathway (Fig. 2). Planar Cell Polarity (PCP) is the alignment of cell orientation along a tissue plane through asymmetric distribution of components of the PCP pathway. Several core proteins have been identified to be of significant importance in this pathway, including the multi-transmembrane protein Frizzled (Fz), Vangl2 and Disheveled (Dsh). A key

feature of PCP is the asymmetrical localization of the core PCP proteins, which occurs through cell-cell signaling gradients. Wnt5a, a secreted glycoprotein, has been found to provide the directional cues needed for this asymmetry to be established. A role for Fibrocystin (FPC), the PKHD1 gene product, has also been suggested, though little is known about its role in noncanonical Wnt signaling.

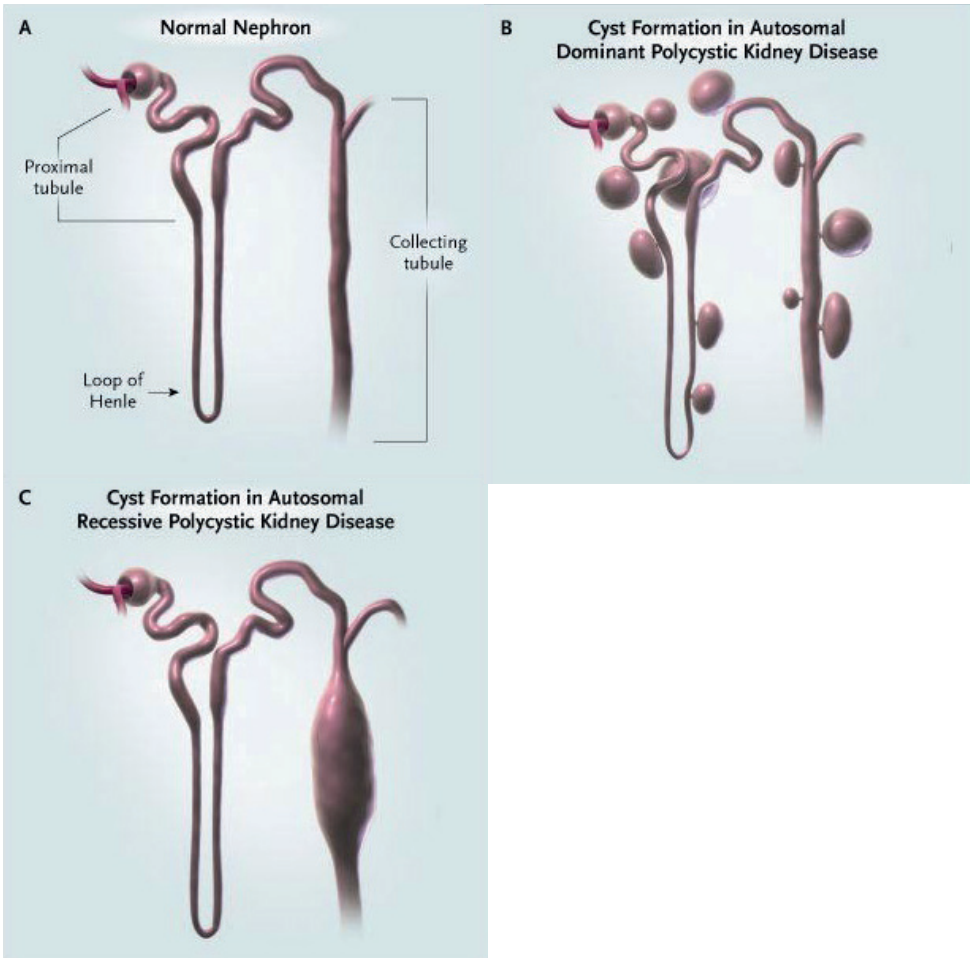


Figure 1. Mechanism of cyst formation in PKD

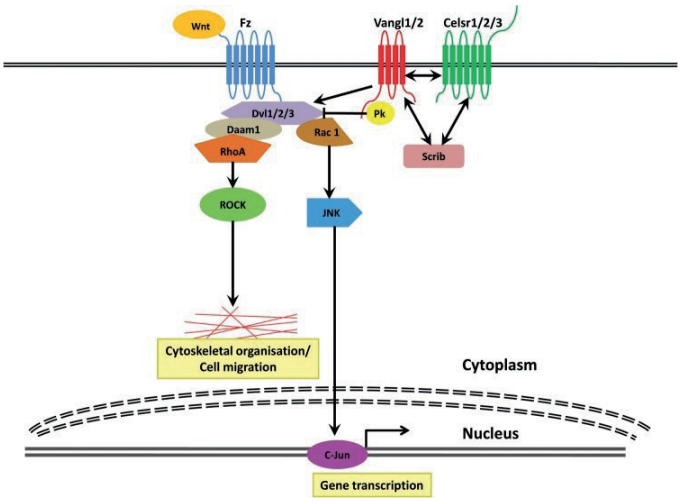


Figure 2. The noncanonical Planar Cell Polarity pathway.
Papakrivopoulou, E., Dean, C., Copp, A. and Long, D. (2013). Planar cell polarity and the kidney. Nephrology Dialysis Transplantation, 29(7), pp.1320-1326.

Discovering new pathways

An important part of my research was to elucidate the role of FPC in the basic biological process of PCP and noncanonical Wnt signaling, with the overall goal of defining the pathway in which fibrocystin works. Human Embryonic Kidney (HEK) cells were used to study protein interactions between Fibrocystin and the core PCP proteins. Additionally, we investigated similar possible protein interactions for Polycystin (PC1), the PKD1 gene product, to see if certain interactions were ARPKD specific or more generally found in PKD. In order to study specific proteins, the HEK cells were transiently transfected with plasmid DNA encoding for the proteins in question. A technique called co-immunoprecipitation (co-IP) was used to study potential interaction. An antibody directed against the epitope tag of a known protein (HA for FPC and PC1, FLAG for Vangl2, V5 for Wnt5a and GFP for Dvl2) was then used to isolate that specific protein from a solution. However, if another protein interacted with this protein (i.e. a ligand), it was pulled down as well, allowing it to be detected in complex with the originally targeted protein.

Following this approach, several proteins were found to interact with both FPC and PC1. First, the interaction of both FPC and PC1 with Vangl2 was examined. We found that Vangl2 clearly interacts with both FPC and PC1. To confirm this is a genuine interaction rather than coincidence, we performed the reciprocal IP in which FPC and PC1 were targeted. Likewise, Vangl2 was now clearly pulled down with both FPC and PC1, confirming this interaction. A similar approach was used to investigate the interaction between FPC or PC1 and another core PCP protein, Wnt5a. Wnt5a clearly bound to both FPC and PC1, though it must be noted that a small amount of Wnt5a protein was also pulled down unspecifically. The reciprocal IP, in which Wnt5a was targeted and FCP and PC1 came down with it, confirmed the interaction between those proteins. Finally, we tackled the question whether a direct relationship between FPC and/or PC1 and Dvl2 exists. Dvl2 is normally located inside the cell, but has been found to move to the cell surface upon Wnt activation. Acting as a signaling hub downstream of Wnt activation, it functions as a switch between the canonical

and noncanonical Wnt signaling pathway. It appeared as if Dvl2 was interacting both with FPC and PC1, however Dvl2 was also pulled down unspecifically, leaving these results hard to interpret. The next step would be to include more stringent washing steps of the IP's when repeating the experiment in order to minimize nonspecific binding.

More possible protein interactions between FPC and PC1 still have to be investigated. However, this is a first step in understanding the cellular pathways that affect cyst formation, which will ultimately help us to develop targeted therapies for Polycystic Kidney Disease.

Elucidating the functional relationship between planar polarity, ARPKD and ADPKD via Vangl2

Besides studying protein interactions, we have been looking into cell-cell adhesions, more specifically the adherence junction. Preliminary data from the Somlo lab has shown that FPC and Vangl2 are working in a pathway which functionally interacts with PC1. Re-expression of Vangl2 in mice with a mutation in PKD1, reduced cyst formation and partially rescued the kidney phenotype (Fig. 3). In order to understand the protective mechanism behind this, a heterozygous (PH2) PKD+/- and homozygous (PN24) PKD1-/- cell line derived from mouse models were used to study this question in a simplified manner. Instead of transiently transfecting the cells, electroporation was used to create stable Vangl2 overexpressing cell lines. By immunoprecipitating Vangl2, we found an interaction between Vangl2-FLAG and E-cadherin, which is confirm results published in the literature. E-cadherin belongs to the family of cadherins and is an important cell adhesion molecule in epithelial adherence junctions. Interestingly, E-cadherin levels appeared to be elevated in Vangl2 overexpressing cells. To further investigate the effect of elevated E-cadherin levels, we performed a functional cell dissociation assay. Equal numbers of PH2 and PN24 cells with and without overexpression of Vangl2 were compared based on cell adhesion capacity after treatment with trypsin and calcium for 1 hour. More cell clusters were seen in PH2 cells compared to PN24 cells, implying more cell-cell association in these cells. However, overexpression of Vangl2 in both PH2 and PN24 cells considerably limited dissociation, as neither PH2 or PN24 cells with Vangl2 could be dissociated off the plate nor each other. This suggests that overexpression of Vangl2 strengthens the cell-cell junction, possibly due to increased E-cadherin. An increase in E-cadherin levels could therefore be a possible explanation for the observation that overexpression of Vangl2 slows down disease progress in PKD1-/- mice. The next step would be to translate this observation made in cells to the animal model.

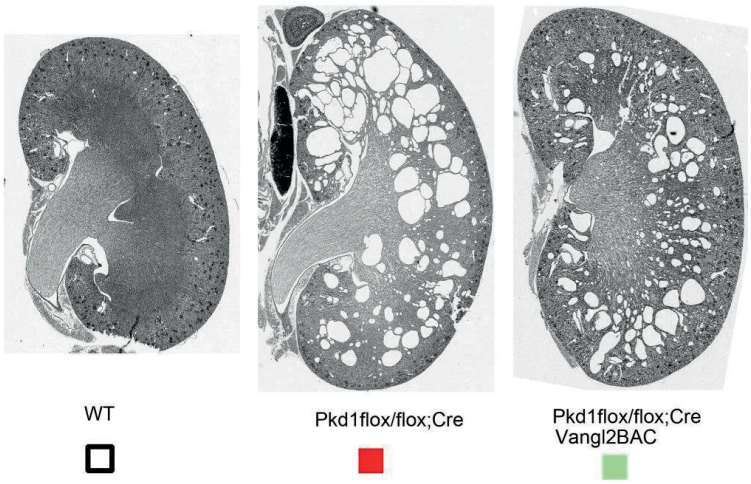


Figure 3. Overexpression of Vangl2 in Pkd1-/- mice rescues kidney phenotype.

Summary

The noncanonical Wnt/Planar Cell Polarity signaling pathway has been found to play a significant role in cystogenesis. A role for Fibrocystin has also been suggested, however its exact role has yet to be elucidated. We therefore aimed to investigate which proteins it interacts with, with the aim to discover the pathway it works in.

Protein interaction studies have been performed between FPC /PC1 and Vangl2, Wnt5a and Dvl2, which are all classified as core Planar Cell Polarity proteins. Both FPC and PC1 appear to interact with Vangl2 and Wnt5a, suggesting they work in a common pathway. A second focus was based on the discovery that overexpression of Vangl2 in Pkd1-/- mice reduced cyst formation and slowed down disease progression. We found that Vangl2 interacts with E-cadherin, which is commonly described in literature. We also found that E-cadherin levels were higher in the Vangl2 overexpressing cells. Cell dissociation assays confirmed that the adhesion capacity of the Vangl2 overexpressing cells appears to be increased compared to non-Vangl2 overexpressing cells, suggesting that increased E-cadherin could be a possible explanation for the ability of overexpressed Vangl2 to improve the kidney phenotype.

More experiments need to be done in order to fully discover how cysts form in PKD. Ultimately, understanding the cellular pathways that affect cyst formation will help us to develop targeted therapies for Polycystic Kidney Disease.

Acknowledgements
I would like to thank Anna-Rachel Gallagher (PhD) for her excellent mentorship during my stay at Yale University. I have gained a tremendous amount of knowledge about Polycystic Kidney Disease and I am proud to have been able to contribute to her research into this disease. Furthermore, I would like to thank Eric Verschuren for teaching me the basic laboratory skills and preparing me for my trip. Finally, my gratitude goes out to Stefan Somlo (Department of Internal Medicine, Nephrology at Yale University), Joost Hoenderop (Department of Physiology, Radboud Institute for Molecular Life Sciences) and the Radboud Honours Programme Medical Sciences for granting me this amazing opportunity.

Elmer Rutjes

(Enschede, 1997)
Bachelor of Medicine 2015-2018

Honours internship:	Department of Geriatrics/ Alzheimer centrum, Radboudumc; Cerebrovascular laboratory, Institute for Exercise and Environmental Medicine, Dallas, Texas, USA
Supervisors:	Dr. Jurgen Claassen; Dr. Rong Zhang, Dr. Evan Pasha

My Radboud Honours Programme experience started after receiving an invitation to an introductory lecture. At first, I doubted whether or not I should apply for the programme, because I was not sure if I would be able to keep up with my regular study. Despite these doubts, I decided to apply anyway. The doubt quickly turned into motivation and this resulted in me learning more than ever before; during both the Honours programme and my regular study. Therefore, I can say I didn't regret this decision for a second and I recommend it to anyone, even if you still have doubts.

I learned a lot during the part of the programme that took place in the Netherlands. During the frequent English presentations in addition to the English proficiency course, I got more comfortable with the language and with giving a presentation. The rotations in the different research institutes gave me a better understanding of my own preferences concerning research and got me more motivated to pursue a PhD.

However, the most valuable experiences came from the internship in Dallas. Meeting a lot of new and interesting people, pushing my boundaries on multiple levels, and experiencing a different culture for a couple of months is a once in a lifetime experience. I felt welcome from the moment I walked through the door and quickly received excellent supervision on both my project and work on the ongoing study. Dr. Zhang didn't only help me learn about research, but he inspired me to bring passion into research, my career and life in general. Even though the weather can be brutal in Dallas, (45°C in the summer), it is an amazing city to live in with a rich culture, excellent career opportunities, great food, and of course rodeos for the enthusiast.

Alzheimer's Disease: incurable, but preventable?

Elmer Rutjes

Many people know a neighbour, a friend or a close relative that has suffered from the awful condition we call Alzheimer's Disease (AD). It is a horrible death sentence you wouldn't wish upon your worst enemy because people with AD lose what many people hold most dearly: their ability to perform simple everyday tasks, memories of loved ones and even their very own sense of self. We don't have a complete picture of what causes AD, and therefore this problem keeps thousands of researchers and clinicians across the globe busy every day. However, we do have multiple hypotheses about the causes of AD and understanding these hypotheses is fundamental in creating a plan for possible strategies to treat and prevent AD in the future. This article will describe some ideas and results from the study we conducted.

The Alzheimer's Disease problem

AD is the most common subtype of dementia and is highly associated with increased age. In fact, 1 out of 5 people above the age of 65 will develop dementia and this goes up to 2 out of 5 above the age of 90. Researchers estimate that the number of people with AD in the Netherlands is around 200,000 and they expect this to double over the next 20 years. Clinicians and researchers consider AD to be a disease with one of the highest disease burdens for the patient. This grim outlook is aggravated by the immense pressure AD places on the patient's caregivers and the national health care system, for example, 54% of the caregivers say that they are under high amounts of pressure and 4% can't handle it much longer. All these problems are reason enough to put in the effort to find ways to reduce the future strain of AD on society.

Hypotheses and progression of Alzheimer's Disease

The hallmark of Alzheimer's disease is the deterioration of memory in combination with the presence of the amyloid β protein in the brain and is diagnosed with multiple cognitive tests for memory and executive functions. Amyloid β is a protein that is present in every human body. However, in AD patients it accumulates into plaques when there is an abundance of the protein. These plaques impair the function of the neurons in the brain and impose an inflammation response of specific immune cells in the brain, which exacerbate damage to the neurons in the brain. The combination of the amyloid β plaques and the glial cell response will over time result in a loss of brain matter and therefore a loss of brain function.

Vascular dysfunction is another likely piece in the puzzle of amyloid β accumulation, as it induces amyloid beta production and leads to faulty clearance. Reduced brain blood flow caused by poor brain vascular health can result in brain damage and in turn upregulate the production of amyloid β. This is similar to blocking the drainage in your sink while

turning the faucet completely on. The water, comparable to the amyloid β, is bound to accumulate at an accelerated rate in the sink, or in the case of AD, aggregate and form plaques in the brain.

Risk factors for Alzheimer's Disease

There are many risk factors that increase the chance of developing AD and it is critical we understand these elements thoroughly. Risk factors can be divided into two main groups: unmodifiable and modifiable risk factors. The unmodifiable risk factors are factors you have no control over, for example, your age, genes and sex. Modifiable risk factors are health gauges that can be influenced by lifestyle, for example your blood pressure, amount of exercise, and smoking.
Researchers Dr. Barnes and Prof. Yaffe say that up to half of all AD cases are potentially caused by modifiable risk factors, which means that we could have a major impact on the prevention of new AD cases. Another vital prodromal condition is mild cognitive impairment (MCI). These patients have an increased risk of AD, and were the patients we examined in this study.

Measuring amyloid ß and arterial stiffness

Arterial stiffness relates to the stretching of the vessel during pressure differences. Arterial stiffening is part of the normal aging process as long-term blood pressure pulses decrease the elasticity of the artery, as the elastic proteins are replaced with stiffer collagen. However, there are many mechanisms that can accelerate normal arterial aging. The most import modifiable risk factors for arterial stiffness are hypertension, smoking and a lack of exercise. One of the most important aspects of arterial stiffening is that it impairs the ability of the aorta to decrease the pulsatile nature of blood ejected from the heart, and create a continuous blood flow as shown in figure 1. This function is known as the Windkessel-effect. A reduced Windkessel-effect causes the blood pressure pulse to be more powerful, which might result in damage to end-organs, such as the brain, in the long run. Arterial stiffness may be linked to AD because it is known to cause vascular dysfunction and remodeling in the brain and impair amyloid β clearance.

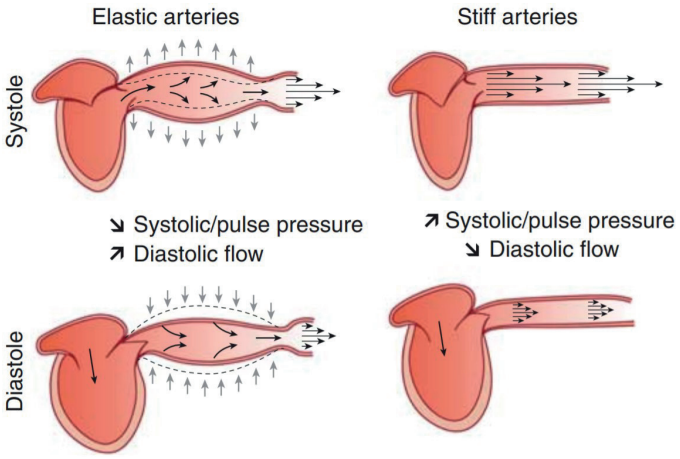


Figure 1. Schematic representation of arterial stiffness and its role in securing blood flow in the peripheral circulation.

Our study observed two specific types of arterial stiffness and their relationship with amyloid β burden in the brain. The carotid β stiffness index, which represents the ability of the carotid artery to stretch during diastole and systole; a higher distensibility means a less stiff artery. We also examined the carotid-femoral pulse wave velocity (cfPWV) which represents the speed at which the blood travels through the aorta, where a higher cfPWV indicates a stiffer artery. These arterial stiffness measurements were analyzed for their association with the amyloid β presence in the brain.
The amyloid β burden was measured with a Positron Emission Tomography (PET) scanner and a radioactive tracer called 18-Florbetapir. This is a complicated imaging modality as it uses radioactive material as its method of signal induction. Radioactive isotopes are attached to proteins that have an extremely specific affinity for certain molecules inside the human body, for example, specific tumor proteins, highly metabolically active cells and in this case, the amyloid β proteins. Every 18-Florbetapir molecule has the potential to give off a signal, and this means that because of its high affinity for amyloid β, a higher signal translates to a larger amount of this protein. The value we used is the Standardized Uptake Value Ratio (SUVR), which represents the signal affected regions versus normalized regions. We calculated the average SUVR of multiple brain regions to create the mean cortical SUVR as depicted in figures 2 and 3.

Results

We found that age was highly correlated with amyloid β presence in the brain, and with both arterial stiffness measures. This is in accordance with previous research and shows that there is likely to be a normal aging process occurring in our study group.

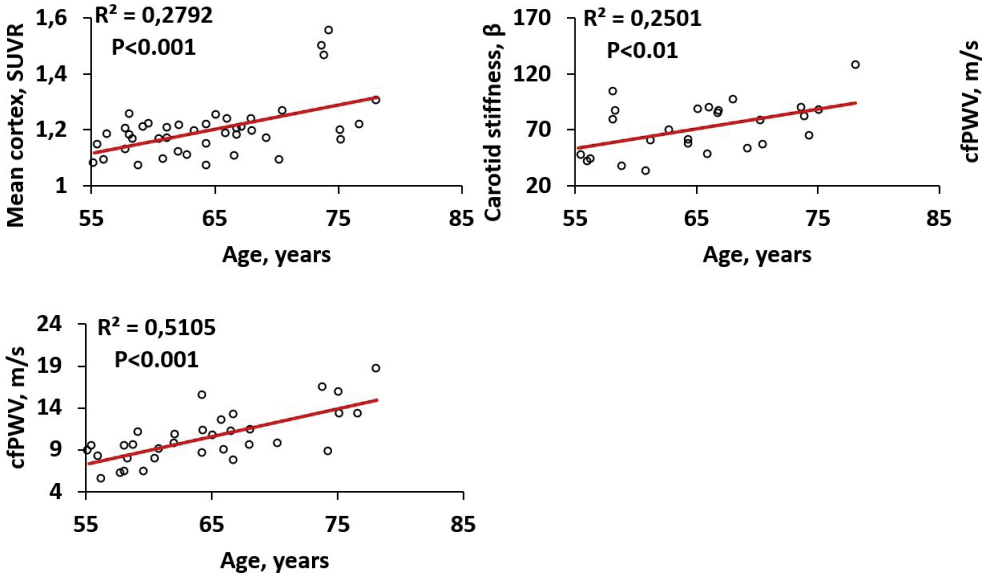


Figure 2. Age in relation with Left) mean cortical SUVR Middle) carotid ß stiffness and Right) cfPWV

We ran partial correlations to determine the relationship of stiffness measures with amyloid β burdens independent of age, sex, genetic vulnerability, body mass index,

education and blood pressure. We found strong associations with both arterial stiffness and amyloid β as seen in figure 3, however, only the carotid β stiffness was statistically significant. This indicates that carotid stiffening might have a bigger impact on the amyloid β burden than aortic stiffness. This finding identifies a potential modifiable treatment target for the prevention of amyloid β accumulation, and perhaps AD in the long run.

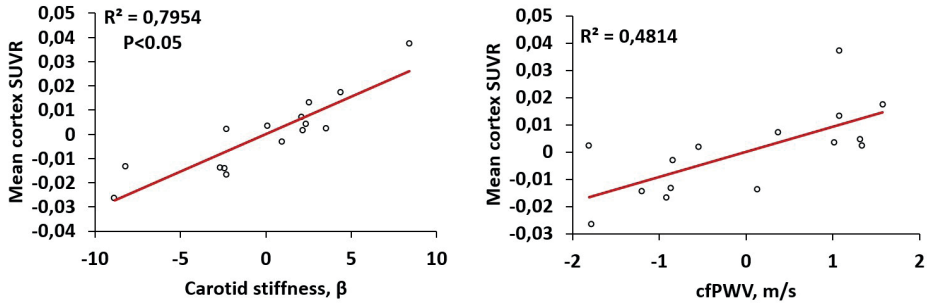


Figure 3. Mean cortical amyloid β burden in relation with Left) carotid stiffness Right) aortic stiffness.

Conclusion and implications

In conclusion: We know that amyloid β is highly associated with AD development, but we do not know everything that influences the amyloid β. However, we are getting closer to unravelling this mystery. We know that cardiovascular risk factors play a large role in the vascular hypothesis and that arterial stiffness might be an important risk factor to control intensively in the future. Researchers have shown that arterial stiffness is partly reversible with extensive exercise, smoking cessation, and controlling both blood pressure and cholesterol. What this study showed is that arterial stiffening is related to amyloid β burden and that monitoring localized carotid stiffness might be more relevant to brain health than the central aorta stiffness. Therefore, a viable approach to treating the devastating condition known as AD is to focus on primary and secondary prevention strategies. To support carotid artery stiffening as a causal mechanism of AD, new longitudinal studies need to be done that examine the effects of risk factor control.

Special thanks to
I would like to thank Dr. Jurgen Claassen and Dr. Marit Sanders for their excellent guidance during my preparation period in the Netherlands. I learned a lot about conducting clinical research and about the different hemodynamic measurements. I want to thank Dr. Rong Zhang for welcoming me into his research team and allowing me to help in the ongoing rrAD study. I would like to thank Dr. Evan Pasha and Dr. Tsubasa Tomoto for helping me with my project and teaching me a lot. I want to thank the rest of the rrAD team for their patience and effort in helping me work on the study.

"As a first year medical student you don't get to take part in research that much and I wanted to explore what it meant to combine a career as clinician with that of a researcher"

Marc Oppelaar

(Tiel, 1996)
Bachelor of Medicine 2015-2018

Honours Internship:	Paediatric Pulmonology, Radboudumc; Translational Medicine, Research Institute of the Hospital for Sick Children, Toronto (ON), Canada
Supervisors:	Prof. F. Ratjen (M.D., Ph.D.) and Dr. J. Roukema (M.D., Ph.D.)

I joined the Honours programme over two years ago to gain more experience in medical research. As a first year medical student you don't get to take part in research that much and I wanted to explore what it meant to combine a career as clinician with that of a researcher.

During my internship in both the Radboudumc and the paediatric hospital SickKids in Toronto, I have definitely developed an interest in a career like this. Being part of one of the largest paediatric research institutes worldwide has allowed me to experience how research can change medicine and what doing research actually means. I also found out that research can be horribly tedious, but the thrill you get when you find something worthwhile totally makes up for it.

But doing research wasn't the only thing which made the experience worthwhile. Being in an unknown city, in an unknown foreign country, is an experience on its own. While living there you have to find a new home in a completely different environment than you're used to. I had responsibilities in my work I had never had before, and outside of work I had to adjust to a completely different city, with completely different people. All this makes an internship abroad not just a good place to develop affinity with research, but also to develop your own personality and skills.

Even though I am nowhere near working as a clinician or researcher, I feel like the Honours Programme has allowed me to experience medical research in a way I wouldn't have been able to otherwise. I am very grateful for this experience, the opportunities I have received at SickKids, and the people I have met. All in all, I can only say that I have had an amazing time in Toronto, learned a lot and would definitely do it again.

New ways to measure lung function in cystic fibrosis

Marc Oppelaar

Cystic fibrosis (CF) is one of the most common recessive genetic disorders we know. Patients with CF are unable to create a certain protein called "Cystic Fibrosis Transmembrane Regulator (CFTR)". This protein sits on top of our cells and regulates the stickiness of our mucus. When the protein doesn't work our mucus becomes thick and sticky. Having this thick and sticky mucus leads to chronic inflammation and mucus-plugs, which in turn damages organs like the lungs, pancreas and intestines. As the years pass, the damage increases until eventually the lungs are unable to keep working as they should. This makes CF an incurable disease with a prognosis of roughly 35 years.

Although this sounds very demotivating, there is good news too! In the past 10 to 15 years, enormous leaps have been made in the field of CF healthcare. New protocols and drugs have been discovered and by using these we have been able to greatly improve lung function in CF. At present, it is expected that life expectancy of CF patients might increase to 50 years or even higher. However, most CF patients still die because of lung problems. Pulmonary care is therefore an extremely important topic in CF care, and monitoring the lung function of children with CF is a huge part of this.

The problem

The way in which lung function is currently measured in CF is usually with spirometry. During spirometry patients have to blow really hard through a tube. The machine then measures how much air is exhaled in one second. The more air you can exhale, the healthier your lungs are.

This seems to be an easy task, but problems arise when we perform this test in young children. Because of our new treatments and protocols, lung function in young children with CF has greatly improved. The improved lung function means that spirometry is now no longer sensitive enough to accurately measure lung damage. When a child with CF performs a spirometry test, it often gives a value which we would expect in a healthy child. This sounds really good, but when we perform a CT-scan we can still see that lung damage is present. We can't routinely perform CT-scans because of radiation damage, but we still need some way to measure lung function more precisely. Another problem is that young children often find it really hard to sustain forced expiration, which is required for spirometry. In short, in young patients with CF we can't really measure lung function, because spirometry is not sensitive enough and too hard to perform for this age group.

The alternative

Luckily, the minds behind CF research have found another way to measure lung function which is a lot more sensitive to lung damage than spirometry. The technique they

found has existed since the 1960s, it just never became popular because the computer technology wasn't available to perform the necessary calculations. But now, decades later, computer science has skyrocketed and multiple machines have been designed to perform this so-called Multiple Breath Washout (MBW) technique. MBW is a technique which simply requires patients to calmly breathe through a tube, just like they are snorkelling. When doing this, patients only breathe in oxygen, which – breath by breath – washes out the nitrogen inherently present in their lungs.

The main outcome of the MBW technique is the "Lung Clearance Index (LCI)". The LCI indicates how good our lungs are at washing out the nitrogen; the quicker the nitrogen is washed out, the better the lung function. This technique has already been shown to be feasible to perform routinely in children and to be very sensitive in measuring lung damage.

MBW in children aged 2-5 years old

However, the age group of 2-5 years old again poses new challenges. To get a good LCI measurement, it is important that during the whole test the patient breathes calmly, with a stable breathing pattern and without moving their lips. For us adults, this seems easy, but for young children this can be challenging. Being in an unknown environment, seeing and hearing the machine, and having to breathe through a tube are all examples of factors which can be stressful to young children. Furthermore, the test takes several minutes and trying to keep a three year old seated during this time without moving their head or lips can be challenging on its own. Because of this, changes in breathing patterns occur regularly in young children and testers often have to repeat the test multiple times to get a good value. This also takes time which is obviously detrimental to a child's concentration. All in all, it can be particularly challenging and time-consuming to perform these tests in this age group.

Alternative MBW outcomes

However, during a MBW test it is possible to measure a lot more than just the LCI. To tackle the problems above, three alternative MBW outcomes have been suggested instead of the LCI: the "shortened LCI", "moment ratio 1" and "moment ratio 2".
The shortened LCI is basically the same as the normal LCI but takes less time. This makes it easier for young children to achieve, seeing that having to be concentrated for two minutes is a lot easier than for four minutes. The downside of the shortened LCI is that it has previously been found to be less sensitive than the normal LCI.

Moment ratio 1 and moment ratio 2 are two parameters which, simply put, show when the majority of the nitrogen is washed out during the test. If this is at the beginning of the test the lungs are healthy, if this is more skewed towards the end of the test the lungs are less so. The good thing about moment ratios is that they are less affected by unstable breathing patterns than the LCI is. However, moment ratios are very hard to calculate and interpret, and fully explaining this is far beyond the scope of this article.
Since MBW has only regained interest in research for a decade or so, not much is known about these alternative outcomes. Using these with young children might increase our knowledge on them, and might help us find a solution to the problems we face when performing MBW in young children. If these alternative outcomes prove to be easier to perform in young children, while also providing us with the same information, then it might be more beneficial to use these outcomes instead.

What did we find?

During my internship I studied alternative MBW outcomes in: (1) their ability to distinguish between health and disease; (2) their ability to track disease progression; (3) their variability; and (4) their values during pulmonary symptoms (i.e. cough, wheeze, exacerbations). These are all themes of which a lot is known the normal LCI, but not about the alternative outcomes.

Our main results were that alternative outcomes are robust parameters that offer an equal amount of information as the normal LCI does. This might not sound too exciting, but more implications can be drawn from this conclusion. For example, if the shortened LCI takes less time to achieve and offers the same information as the normal LCI, would it not be favourable to use it in this specific age group? Using the shortened LCI then clearly seems to be beneficial.

Benefits are less obvious in using the moment ratios. We found that these outcomes offered the same information as the LCI, but we found no clear benefit of using these outcomes in our population instead of the normal LCI. Their complicated method of measurement and interpretation only made them seem less favourable to use. However, this was true only for our specific population. With our findings we gathered evidence that these outcomes might be more useful in, for example, older populations or interventional trials.

Wrapping up

Our study is one of the first which investigated alternative MBW outcomes in a number of different themes and in one setting. This makes our results very valuable, but because there's little other evidence to back up our claims, we should be careful drawing conclusions. So what happens now? Well, alternative outcomes won't immediately make it into clinical practice. The normal LCI hasn't even gotten that far yet and we need to gather much more knowledge to make solid claims. What might happen is that alternative outcomes will now receive more interest since their usefulness has now been demonstrated. We might start seeing more researchers reporting these outcomes in their articles and therefore more knowledge will become available. This is something which will need to happen before we can really start to rely on these alternative outcomes, and before they become useful in clinical practice. Our study has therefore significantly contributed to our scientific understanding of MBW, and hopefully has sparked interest for future research.

Special thanks to
I would like to thank Prof. Dr. Felix Ratjen, Michelle Klingel and Renee Jensen for their perfect guidance during my project. I am very thankful for the opportunity and knowledge they have given me and could not be more grateful for their help and support. I am also thankful for Dr. Jolt Roukema for teaching me about cystic fibrosis, making me excited about clinical research and giving me this opportunity.

Iris Teunissen van Manen

(Wageningen, 1996)
Bachelor of Biomedical Sciences
2015-2018

Honours Internship:	Department of Laboratory Medicine, Radboudumc; Anne McLaren Laboratory of Regenerative Medicine, University of Cambridge, UK.
Supervisors:	Prof. D. Swinkels, Prof. L. Vallier and Dr. R. Tomaz

At the start of the Honours Programme, I was not quite sure what to expect. I was really interested in biomedical research and wanted to focus more on this during my bachelor's degree. I knew that the honours program would give me the opportunity to get acquainted with all the different research fields within the Radboudumc, and that I would be optimally prepared for an internship abroad where I could focus on a specific research topic. However, I did not know whether the extra hours would be feasible for me and if going abroad was the right step.

About a year ago, I was introduced to professor Dorine Swinkels, who gave me the opportunity to work on a new project within Radboudumc's iron research team. Within this project, it was my goal to make liver cells from patients with an inherited iron disease from patient-specific stem cells. Because this method is not yet applied in Nijmegen, I travelled to Cambridge for this, which was one of the places I was really eager to visit. However, as I am not the typical traveling type, going abroad for 5 months seemed quite challenging for me.

Now that I have finished this internship, I can say that I have learned more than I had ever hoped for. Living and working in Cambridge has been a great experience for me. In the stem cell laboratory, I have learned about all aspects of scientific research while working with welcoming and experienced colleagues and supervisors. In addition, exploring Cambridge's unique culture is something I will never forget. I am therefore very glad for my decision to participate in the honours program and I am grateful for all the opportunities that I was given.

Can we use stem cell-derived liver cells to diagnose IRIDA?

Iris Teunissen van Manen

Chances are you have never heard of Iron Refractory Iron Deficiency Anaemia, or IRIDA in short. As the name implies, this rare form of anaemia can't be treated by swallowing iron tablets. The reason for this can be found in the origin of the disease, the liver. There, the transcription of the hepcidin gene (HAMP) is regulated via the bone morphogenetic protein 6 (BMP6) pathway. This pathway is active when the concentration of iron in the blood is too high. The production of hepcidin will help lower the amount of iron in the blood by blocking the transport of iron from our food to our bloodstream. Like most molecular pathways, the production of hepcidin can also be lowered by a negative feedback mechanism. This is necessary when the amount of iron in the blood becomes too low. The matriptase-2 protein, located on the membrane of liver cells, is thought to provide this negative feedback by cleaving off the BMP6 coreceptor hemojuvelin (HJV). As shown in Figure 1, this is exactly where the cause of IRIDA lies. IRIDA patients have a genetic mutation in the gene that codes matriptase-2, which is called TMPRSS6, causing ineffective HJV cleaving. Unable to inhibit the production of hepcidin in their liver, IRIDA patients are constantly blocking their uptake of dietary iron. Eating more spinach or swallowing iron pills will therefore typically not improve this chronic anaemia, which is why these patients require frequent blood transfusions.

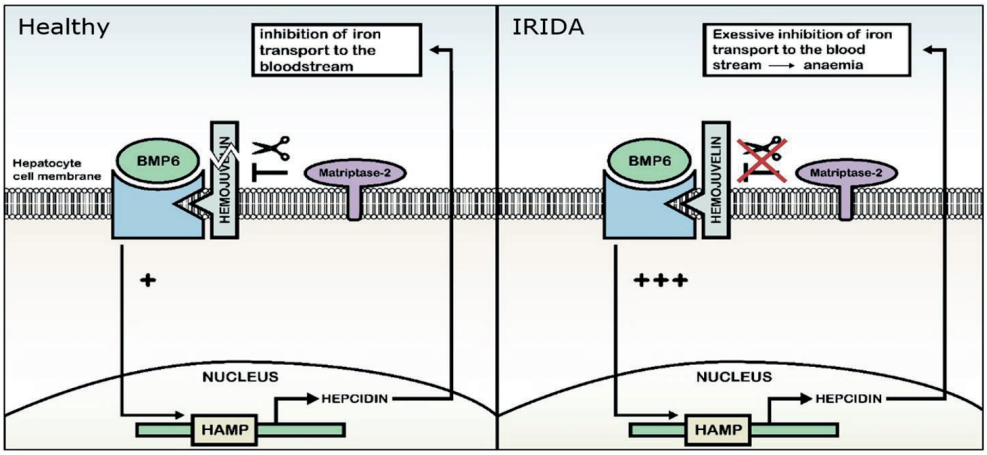


Figure 1. The current theory about how the expression of hepcidin is regulated in healthy persons, and how this is affected in IRIDA patients.

Diagnostic challenges

Anaemia is a very common problem and can have many different causes. This complicates the diagnosis of rarely occurring diseases like IRIDA. When a patient has low iron and high hepcidin concentrations in the blood, the TMPRSS6 gene can be analysed to search for mutations that cause IRIDA. The problem, however, is that in many cases we do not know whether a certain TMPRSS6 variant causes the disease or not. To prove that a genetic variant results in the disease, cellular models are needed. In the past, these models consisted of cells with an extra piece of DNA that contained the TMPRSS6 gene with the patient's mutation. However, as these are not the patient's own cells, we cannot be certain that these models reflect the situation in the patient.

So how can we make a cellular model that is patient-specific? As mentioned before, the origin of IRIDA lies in the liver. This means that a cellular model for IRIDA should contain liver cells, also called hepatocytes, which are not easily obtained. Fortunately, it is now possible to produce hepatocytes using induced pluripotent stem cells (iPSCs). These are stem cells produced from adult cells that have undergone reprogramming or dedifferentiation after being exposed to a cocktail of specific embryonal factors. Just like embryonic pluripotent stem cells (ePSCs), iPSCs can differentiate towards many specialized cell types. Moreover, when iPSCs are obtained from a patient with IRIDA, both the iPSCs and the hepatocytes produced by differentiating these iPSCs will have the patients' DNA. These iPSC-derived hepatocytes could therefore be used in cellular models to study the impact of a specific TMPRSS6 variant.

Taking the first steps

This is the first time research to IRIDA has involved stem cells. It is therefore important to first investigate if hepatocytes derived from stem cells are suitable cells for a diagnostic model. IPSCs are differentiated into hepatocytes by mimicking the embryonal development of the liver. This means that the cells are gradually exposed to different mixtures of signalling proteins. Ideally, this should result in perfectly mature hepatocytes. Unfortunately, this is not exactly the case. Even though the protocol for differentiation has been optimised many times, the resulting cells will always have some embryonal features. This why these cells are also called hepatocyte-like cells, or HLCs. Measuring the expression both liver-specific and IRIDA-related genes is therefore crucial.

With the future goal to use these cells for diagnostic purposes, it is also important to make sure that cells from IRIDA patients can be distinguished from healthy ones based on IRIDA characteristics. So how can we do this? Previous research has found that the production of hepcidin is increased when BMP6 binds to its receptor on the liver cell. This should also be measurable in the HLCs. When BMP6 is added, the hepcidin production should increase in cells from both healthy controls and IRIDA patients. However, the HLCs from the IRIDA patients lack effective inhibition of this process. We would therefore expect a higher hepcidin production after BMP6 stimulation in the patient HLCs when compared to the control. To test this theory, we have differentiated the iPSCs from one IRIDA patient and two healthy controls into HLC. Besides measuring the expression both liver-specific and IRIDA-related genes, we have measured the hepcidin production in the HLCs before and after the addition of BMP6. With this approach, we hoped to find that HLCs from the IRIDA patient differ from the control cells based on IRIDA characteristics.

Differentiation of the stem cells into liver cells

To make patient-specific hepatocytes, the iPSCs from the patient and controls underwent a 30-days differentiation process. At the end, the cells should have looked and behaved like fully-grown liver cells. However, after we started the differentiation of the stem cells, it became clear that many cells died during the first stages. Because of this, we were left with only few plates of cells to compare at the end of the process. The reason that so many cells died is still unknown, however, the surviving cells seemed to grow according to plan and appeared liver-like at the end of differentiation. To prove that the cells also behaved like liver cells, we performed several experiments. For instance, we measured the expression of liver-specific genes and visualised the presence of liver-specific proteins. Even though we should note that we found varying outcomes between the different experiments and cell groups, we can conclude that the cells indeed showed liver-specific features. Therefore, we could continue to investigate the IRIDA characteristics in these cells.

Stem cells to model IRIDA

To make sure that the HLCs express IRIDA-related genes, we measured the RNA levels of hepcidin and related genes in iPSC derived, primary and fetal hepatocytes. In agreement with the typical immature feature of HLCs, we found that the expression levels of hepcidin (but also the other measured genes) was more similar to foetal cells rather than adult cells. Nevertheless, we were able to measure expression of these genes in the HLC, which was an important first requirement for the use these cells as diagnostic tools for IRIDA.

The next step was to find out if the RNA levels of hepcidin can also be increased by BMP6. To do so, we incubated the cells with BMP6 and measured the hepcidin expression afterwards. We performed this experiment several times for both the patient and control lines. The results showed a rather large variation in the hepcidin expression between the different experiments. This might be due different qualities of the HLCs, which is closely related to the efficiency of the differentiation. Despite this, we did measure higher hepcidin expression levels in both patient and control cells after adding BMP6. Based on this, we could confirm activity of the BMP6 pathways in our cells.

Finally, we compared the hepcidin expression in the patient cells to that in the control cells. Despite variations in gene expression between the different experiments, the fold increase of hepcidin expression after BMP6 stimulation was consistently higher in the patient HLCs. Although this result does not take other possible interfering factors into account, it is consistent with the hypothesis that the patient cells are not able to inhibit hepcidin expression due to a mutation in the TMPRSS6 gene. As these promising results were found using HLCs during a period of challenging differentiations, we recommend repeating these experiments with higher quality HLCs. This way, it would be possible to validate these results and confirm that HLCs from IRIDA patients will develop IRIDA characteristics. This is a crucial step before we start using these cells for diagnostic purposes.

Conclusion

To summarise our results, we have first found proof that the BMP6/hepcidin pathway is active in iPSC derived hepatocytes. Secondly, our results show that the fold increase of hepcidin expression after stimulation with BMP6 is larger in HCLs derived from the IRIDA patient when compared to healthy donors. This is consistent with the theory that IRIDA patients are unable to effectively inhibit hepcidin production due to their mutated TMPRSS6. As these results give the indication that HCLs can develop IRIDA characteristics, it would mean that HLC from IRIDA patients could indeed be used in future diagnostic tests. However, the different quality of the HLCs caused rather large variations between the experiments. It is therefore our goal to repeat the experiments using an optimised protocol. In addition, we want to explore other options to test IRIDA characteristics in HLCs besides measuring BMP6. This could eventually lead to the development of new diagnostic tests for IRIDA using patient-specific HLCs.

Special thanks to
Prof. D. Swinkels and Prof. L. Vallier for their excellent mentorship and providing me with the opportunity to work on their wonderful project. Dr. Tomaz, for teaching me everything I needed to know about stem cells research.

"The honours programme taught me some key features that are essential for a good researcher: the importance of open and continual communication to solve arising difficulties, the patience and commitment in conducting experiments and the creativity in seeking explanations for mind-boggling events."

Daniël Urlings

(Maastricht, 1997)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Internal Medicine, Radboudumc; Department of Human Microbiology and Immunology, Tel Aviv University, Tel Aviv, Israel
Supervisors:	Prof. Dr. Mihai Netea; Prof. Dr. Nir Osherov

During the first year of the honours programme I got to know my fellow students. I noticed their dedication to study and their discipline in preparation. Together we visited the different research departments of the Radboudumc. It was fascinating to see the differences in how research is conducted and how many different themes are worked on. Along the way I became more aware of my personal interests in research and eventually even managed to arrange my own internship.

The honours programme taught me some key features that are essential for a good researcher: the importance of open and continual communication to solve arising difficulties, the patience and commitment in conducting experiments and the creativity in seeking explanations for mind-boggling events. Moreover, this programme brought me in contact with many inspiring people, which I am grateful for.

The internship at Tel Aviv University was most definitely one of the best experiences I had so far. One of the most vibrant cities in the world, A Mediterranean beach minutes away from my apartment and some of the most appetizing lunches: I truly had an amazing time. The research group was heart-warming and the conversations with Prof. Osherov made me think in new ways. Furthermore, it was interesting to experience the different work culture, with more room for the researcher as an individual, which I think has something to do with the assertive culture.

Apart from the research, the conversations that I had with people about the conflict gave me new insights on how complicated some situations can be. It has taught me how little I knew and how much I can still learn.

Competing with *Aspergillus fumigatus* in a game of dodgeball

Daniël Urlings

Fungi play important roles in many aspects of human life. They play an essential role in the nutrient cycle of ecosystems; they are used by humans in food processing and they have led to the discovery of most antibiotics. Most fungi do not impact our health, and some are even beneficial. However, some fungi can cause illness to humans. Of all disease inflicting fungi, the group called 'opportunistic fungi' causes most concern to physicians and scientists. Opportunistic fungi are known to infect those with a weakened immunity where they can cause invasive infections leading to one and a half million deaths each year. Current environmental changes, extensive use of antifungals in agriculture, and global trafficking accelerate the evolution of virulence and resistance in these fungi. Despite the improvements made in developing new diagnostic tools and antifungal treatment regimens, mortality rates remain unacceptably high. Furthermore, due to increased use of immunosuppressive therapies and a rise in comorbidity rates, the number of immunocompromised people is growing. Therefore, research into new antifungal therapeutic strategies is needed.

Keep your friends close and your enemies even closer

One of the most common opportunistic fungi is A. fumigatus. Each year there are about 200,000 cases of invasive infections caused by this fungus resulting in about 100,000 deaths. Every day we breathe in hundreds of spores derived from this fungus. The spores that enter our lungs are usually swept back out by tiny hairs called cilia. If the fungus manages to penetrate the airway, special immune cells come into play. These cells, alveolar macrophages and neutrophils, recognize, swallow and destroy the invading spores. This process is called phagocytosis and several microbes have been shown to counteract it, for example, by avoiding being swallowed or by defusing destructive compounds. Normally, the immune system has enough reserves to outweigh this microbial resistance. However, when the immune system is weakened these counter-defence mechanisms can lead to a proliferation of the pathogen. Studying the resistance mechanisms of opportunistic fungi against our immune system could lead to the identification of the desired targets for new antifungal development.

A game of dodgeball

Copper may play an essential role in our defence against A. fumigatus. Our immune cells use copper to demolish engulfed microbes. Upon exposure to microbes, the copper levels within our macrophages start to increase. This copper is then transported to the phagosome, the vesicle containing the microbe. Inside the phagosome, copper forms damaging particles and sabotages essential fungal proteins. Several fungi have been found to be in the possession of copper resistance mechanisms. Upon exposure to high copper levels, copper exporters facilitate the elimination of copper and special chaperones bind the

free intracellular copper. This process can be compared to a game of dodgeball. The fungus is out when it is hit by copper thrown by our immune cells. Therefore, it tries to catch the free copper and throw it back, instead of letting it inflict damage. Lately, researchers have started investigating the dodgeball tactics of A. fumigatus. This has resulted in the discovery and characterization of two genes: the transcription factor AceA and the copper exporter CrpA. Copper excess activates AceA, which in turn stimulates the copper resistance response, for example copper elimination by CrpA. However, most parts of the copper resistance machinery have remained unclear. Recently, all genes of A. fumigatus that respond to copper excess were mapped using an RNA sequence study. By analyzing this dataset, several genes were identified that could possibly contribute to the copper resistance machinery of A. fumigatus. This project was set out to reveal the involvement of two of these genes, namely Afu2g09700 and Afu3g07690.

Disarming team fungus

One of the most frequently used ways to characterize the features of a gene is to delete the gene itself. The defects that occur in the obtained mutant can give you a better understanding of the functions of the missing gene. For example, when a gene that codes for a copper chaperone is deleted, it is expected that the capability of the mutant to catch free copper will be decreased. Therefore, comparing the mutant with the original organism under certain conditions can give new insights in the functions of a gene. Analysis of the genetic sequence suggested that both genes function as a copper chaperone. Therefore, it was expected that mutants without these genes would show reduced resistance to copper excess and oxidative stress. To evaluate the copper regulating features of the genes Afu2g09700 and Afu3g07690, two mutants were created using homologous recombination. This is a process in which an artificially made DNA sequence replaces the original gene through genetic exchange.

How to disarm a microbe?

Separate parts of the replacement sequence were created using the polymerase chain reaction (PCR), a technique which makes it possible to amplify parts of DNA. Following this, the Gibson reaction was used to fuse the separate DNA fragments into plasmids, which were then multiplied by bacteria. Thereafter, the plasmids were linearized to allow genetic exchange and then inserted into spores of natural A.fumigatus. After each step in this process products were validated using gel electrophoresis. An antifungal resistance gene was used as a replacement for the gene of interest in each mutant. After genetic transformation occurred all fungi were exposed to this antifungal, allowing only survival of the mutants in which the genetic exchange was successful. Thenceforth, the genetic information of each obtained mutant was checked for proper deletion of the gene of interest. This was done by PCR, replicating the regions in and around the exchange site. Three mutants of the 97 gene met conditions for successful deletion, of which one was used for further testing. Due to impaired spore formation of the 76 gene mutants only one mutant could be isolated for genetic evaluation. PCR verification of this mutant was not conclusive for a correct exchange indicating possible preservation of the 76 gene. However, because it was not possible to repeat transformation in the limited time given for this project, this 76 mutant was used for further testing.

Paralympic dodgeball

The best way to check if loss of the genes weakened A. fumigatus was to simulate a game of genetic dodgeball. Mutants were tested for growth and survival under various conditions of copper and oxidative stress. Oxidative stress is caused by reactive oxygen species, one of the damaging particles formed by copper. Oxidative stress was induced in these experiments using menadione. Furthermore, the performance of the mutants was compared to the performance of unmodified A. fumigatus and a mutant lacking the CrpA gene. The experiments were performed on solid and liquid growth media, and part of the experiments on solid media are presented in Figure 1.

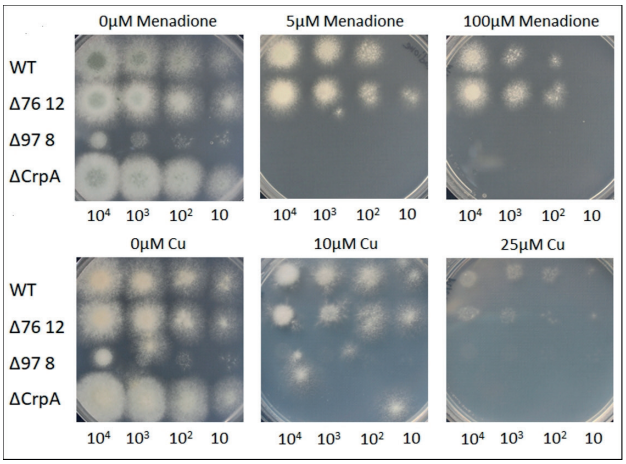


Figure 1. Pictures from MIC menadione and copper plates taken after 48 hours of incubation at 37 degrees Celsius. Indicated number of spores of indicated strains grown on solid MMV medium in the presence of the indicated concentrations of menadione and copper.

The final score

Altogether, mutants of the 97 gene showed impaired growth when compared to the unmodified wild type of A. fumigatus under all conditions, even in the absence of stress inducing agents. Furthermore, mutants were more susceptible to oxidative stress and copper excess, comparable to the CrpA mutant. These results imply that Afu2g09700 contributes to the copper resistance of A. fumigatus. Interestingly, the obtained 76 mutant showed susceptibility patterns comparable to the unmodified wild type, indicating no emerged impairment. However, the mutant used for testing did not show a conclusive sign of successful gene exchange, indicating possible preservation of the 76 gene. The lack of growth and spore formation in the 76 mutants after transformation could indicate that the Afu3g07690 is in fact an essential gene for fungal survival.

Conclusion

This project broadened the understanding of the copper regulon in A. fumigatus. By observing the characteristics of the obtained mutants, it was found that disruption of Afu2g09700 resulted in increased susceptibility to oxidative stress and copper excess. Furthermore, disruption of Afu3g07690 in A. fumigatus resulted in no growth, suggesting that Afu3g07690 could be an essential gene. Therefore, further investigation of these genes may give rise to the identification of the desired targets for new antifungal development.

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"One word: Wow! What a great city Chicago is, and what an experience to live there for 6 months and do such exciting research."

Kiedo Wienholts

(Lichtenvoorde, 1996)
Bachelor of Medicine 2015-2018

Honours Internship:	Department of Surgery, Radboudumc; Department of Surgery, University of Chicago, USA
Supervisors:	Prof. Dr. Harry van Goor; Dr. John Alverdy, Dr. Olga Zaborina

One word: Wow! What a great city Chicago is, and what an experience to live there for 6 months and do such exciting research.

I was very enthusiastic to be linked to the department of surgery at Radboudumc. I was always interested in surgery and was curious about this research. I wanted to see what doing research actually encompassed, and as part of my Honours trajectory I got the opportunity to do this at the University of Chicago. A top-class university that really awed me, though the city itself didn't attract me that much at first. Now that I've been there, I can tell everyone how great and exciting it is!

First of all, the research I was able to do there was truly exhilarating. They really let me do so much independently, so I had to find out myself which experiments to perform. Although they also supported me, I learned so much from this self-sufficient culture in the lab. Now, I can perform mouse surgery, set up relevant experiments to investigate different hypotheses, and carefully consider unexpected results. In particular, actually performing surgery on my own was so appealing and encouraged me to pursue a career in surgery and research.

Then the city itself was amazing. The skyscrapers everywhere which provided a beautiful skyline all over Chicago, Lake Michigan with all its pleasant beaches, the parks and their music festivals, and always so much to do. This is just a tiny part of my memories of Chicago. I really enjoyed it there. Meeting new people, living totally independently, the different culture and mentality in America; all kind of things that I'll never forget, and which have broadened my horizon.

I'm really thankful for this amazing experience and my development at both a scientific and personal level.

The overlooked microbiome in surgery: an easy way to save lives

Kiedo Wienholts

Face transplants, robotic surgery, and seeming miraculous recoveries; surgeons' skills and their equipment are evolving faster than ever, and things that no one thought were possible are actually happening. These milestones in surgery are all amazing, and to know that things are getting even better is great news for everyone. 'Advanced' equipment is becoming even more so, with all kinds of features. Surgery rooms are controlled for everything that could possibly influence an operation. Great developments in hospital care have already saved many lives. However, this focus on increasingly complicated technology has had the unfortunate effect that small, simple but very important areas for improvement are overlooked. These are the so-called 'low hanging fruit' of the surgical world.

One factor in surgery that has been that hasn't been fully explored for so many years is the human microbiome. A complication associated with this has already cost too many lives. We're talking about anastomotic leakage.

What is anastomotic leakage?

During an operation on the intestine, for example when a tumor is removed, it is necessary to reconnect the two loose ends of the gut. Otherwise, the stool that goes through our intestines would leak into our belly, causing a dangerous situation resulting in a lot of pain and eventually death. The act of reconnecting the intestines is called making an 'anastomosis'. This can be extremely difficult as it must be tightly closed, or else it will still leak. Anastomotic leakage is a serious complication but is very common. One in five persons will have an anastomotic leak following this kind of surgery.

For decades, surgeons and researchers have been trying to improve surgical techniques in order to reduce anastomotic leak. New staplers, particular suturing techniques, and special glues have been tested. In addition, the circumstances around the operation are better controlled than ever. But despite all these improvements, the rates of anastomotic leakage are the same as decades ago, and death rates haven't been reduced.

But why haven't we been able to improve this?

Now, the microbiome comes in. The human microbiome consists of all microorganisms such as bacteria, viruses, fungi present on the inside and outside of a human being. More and more evidence is available on how we live together with this community of microorganisms, most of the times in harmony, and how it influences on our health. Living in harmony with all these little germs... wait, aren't they supposed to be dangerous? Well, indeed, some of them can be dangerous for our bodies, we call them pathogens, but they will only become dangerous if they flourish in number or get in the wrong places. Actually, most of the germs in our microbiome compete against pathogens and will help our bodies.

These germs can, for example, break down sugars or other nutrients so we can use them, while otherwise we would just throw them away with our faeces.

The gut in particular possesses a large microbiome with bacteria as its biggest players. Trillions of bacteria are present in our gut with hundreds of different species. They live together with us and will help us throughout our lives. But, when surgery is performed, the environment of all the bacteria in our gut is impacted. This can disturb the balance between pathogens and beneficial germs, giving a helpful shift to the pathogenic bacteria. In this case, they can flourish, and our good germs can't compete against them anymore. The good ones lose power and the pathogens can make you very sick. So, you would think that such an impact on our microbiome must be taken into account when performing surgery, right? It makes sense to try to make the impact as damaging as possible for our pathogens while helping our good germs. However, until recently, this hasn't been done. Now new research has shed light on this issue and how it can be improved, leading to progress in mastering surgeries.

Bacteria can make holes in our gut

For many years, the lab in which I did my internship has been looking at the impact of surgery. The team looked at the microbiome and analyzed changes before and after surgery. They observed anastomotic healing in the presence of different bacteria and published very interesting results. An important thing they noted is that one specific 'good' bacterium, called E. faecalis, is able to flourish at the anastomotic site after surgery. This seems to be a good thing, as it could compete against the pathogens. However, this lab also showed that E. faecalis can transform into an aggressive bacterium when its environment is disturbed during surgery. In these conditions, it was shown that, E. faecalis can break down collagen. This is a very important substance which ensures firmness in all kinds of human tissue. Collagen is present in your skin, your eyes, and also in your gut. Thanks to collagen, these tissues do not come apart; it sticks the tissues together. So, when the bacterium E. faecalis flourishes, transforms and breaks down collagen, it will literally tear the gut apart and make holes in the anastomosis. This is exactly what happens after anastomotic surgery and is a major cause of anastomotic leak.

E. faecalis uses our own body against us

The lab is now investigating the mechanism of how E. faecalis can become so aggressive under surgical conditions and how it breaks down collagen. This is an important part of the research, because if the mechanism is known, it can be targeted with drugs to stop it. We found the following: E. faecalis is actually manipulating a system in our own body to become aggressive and make holes in our gut.

Our body uses lots of different systems and substances to break down and build up tissues every day. For example, our hair is growing constantly; it is built up by our own body. If we get sun-burnt, our body will replace the burned skin. And in the case of a bleeding wound, a blood clot will form and eventually be broken off. This last system is also involved in the virulence of E. faecalis. Under normal circumstances, our body uses a complex system to balance between forming blood clots and breaking them off. This is necessary so a wound doesn't bleed too much, but will also prevent the forming of too many blood clots in our vessels causing infarctions. A critical substance in this

system is called plasminogen. Normally, plasminogen travels in blood in our vessels and is inactive. However, sometime after our body has formed a clot against bleeding, plasminogen will be converted into its active form (plasmin) by a substance released from our vessels. Now, it can accomplish its function: breaking down the clot.

We found that E. faecalis uses this degrading activity of plasmin in breaking down collagen. First, it binds the inactive plasminogen to its surface. Next, it binds another circulating activator of plasminogen, called urokinase, and then the bacterium activates plasminogen. In this way it doesn't need an injured vessel and can activate its plasminogen by just binding circulating substances. Now, E. faecalis has a dangerous wrecking ball on its surface to make those holes in our gut.

Time to save lives!

The mechanism of the plasminogen system in breaking down blood clots has been known and widely described for decades. Therefore, drugs to tackle problems in this area already exist. They are safe, cheap, and are already widely applied in hospitals. One of these drugs is tranexamic acid (TXA). TXA is used in all kinds of surgery to prevent massive bleeding. It blocks the activation of plasminogen and thereby prevents the function of plasmin: breaking down the clot.

We theorized using this blocking function of TXA to prevent the damage caused by E. faecalis, translated our theories into mouse models and confirmed them in lab experiments. By introducing TXA into the gut of the operated mice, we significantly reduced anastomotic leaks. TXA prevented E. faecalis from binding plasminogen and thereby it couldn't activate it. In other words, E. faecalis couldn't transform into its aggressive form. We prevented it from mounting the wrecking ball and it lost the ability to make holes in the gut. Now it's time to translate this mouse model into humans and save lives!

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