7000 liters of blood pump through an average-sized human per day.

60 to 90 heart beats per minute.

2.5 to 3.8 billion heart beats over a lifetime of 80 years.

2 blood circuits
- The large, systemic circulation between the heart and all other body tissue
- The smaller, pulmonary circulation between the heart and the lungs.

Deaths and Hospitalizations:
- Coronary heart disease: 806 hospitalizations, 155 deaths
- Cardiac insufficiency: 465 hospitalizations, 56 deaths
- Heart attack: 266 hospitalizations, 64 deaths
- Valvular heart disease: 96 hospitalizations, 17 deaths
- Congenital heart disease: 28 hospitalizations, 0.6 deaths
- Arrhythmia: 506 hospitalizations, 29 deaths

Data from: Neuer Deutscher Herzbericht 2014, DGK Cardiology

Link: www.med1.mri.tum.de
Heart diseases in total in 2011 (per 100,000 persons)
- 2166 hospitalizations
- 322 deaths

Arrhythmia
- 506 hospitalizations
- 29 deaths

Heart attack
- 266 hospitalizations
- 64 deaths

Cardiac insufficiency
- 465 hospitalizations
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Congenital heart disease
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Illustrations: edlundsepp from data in: Neuer Deutscher Herzbericht 2014, DGK

In an interview with Faszination Forschung, Alessandra Moretti and Karl-Ludwig Laugwitz explain how they develop models of human heart disease from patient-specific stem cells, why the cells serve as software and not hardware for therapeutic use, and how they pave the way for novel treatment options for patients.

Heart Tissue from Stem Cells: A Powerful Avenue for Novel Therapies
Dr. Alessandra Moretti & Prof. Karl Ludwig Laugwitz

**Dr. Alessandra Moretti** was born in Padua, Italy, in 1967 and completed a doctorate in pharmacology and toxicology at the university there in 1997. She worked at the University of California, San Diego from 2002 to 2005, followed by a year at Harvard. Since 2006, this stem cell specialist has been leading a working group for molecular and cellular cardiology at the First Department of Medicine, Klinikum rechts der Isar at the TUM.

**Prof. Karl-Ludwig Laugwitz** was born in 1968 in Berlin, Germany. He studied medicine at the FU Berlin and received his doctorate there in 1996. In 2000, Laugwitz moved as junior professor to TUM’s First Medical Department, before starting as Heisenberg professor of the German Research Foundation at the University of California, San Diego in 2002. The cardiologist then returned to TUM’s Klinikum rechts der Isar in 2006 and has been Director of its First Medical Department since 2012.

Moretti and Laugwitz have each received several awards for their research and they both have a long list of publications in high-impact journals. They have been married since 1996.
**Doctor Moretti, Professor Laugwitz – you produce models of patients’ hearts. What does that actually mean?**

**Moretti:** “Heart” is slightly overstating it – but what we are actually able to obtain with iPS cells from every individual are cardiac muscle cells (myocytes) and heart tissue segments.

**iPS cells?**

**Moretti:** Induced pluripotent stem cells. Like embryonic stem cells, iPS cells can be grown into any cell type in the human body. And we are now able to generate iPS cells even from highly differentiated adult cells. Japanese researchers Shinya Yamanaka (ed. note: 2012 Nobel Prize winner along with John Gurdon) and Kazutoshi Takahashi pioneered this technology first in mice in 2006 and, one year later, in humans. So in 2007, when human iPS cells became possible, we quickly set up the technique in our laboratory. We saw the potential of a transformative novel tool for cardiac research.

**How do you obtain iPS cells?**

**Moretti:** First of all I would need a syringe of blood from you.

Oh dear!

**Moretti** (laughs): Well, ok, just a small syringe – eight milliliters is not a lot. We then isolate immunocompetent cells – T lymphocytes – from your blood and transfect them with the four proteins identified by Yamanaka as sufficient to reprogram differentiated cells into pluripotent stem cells. These “reprogramming factors” are OCT4, SOX2, KLF4 and c-MYC. They are transported into the cells by viruses, which we equip with the genes for the four factors beforehand.

**What happens next?**

**Moretti:** After about a month, iPS cells will be developing randomly in the culture dish. You can identify them by their appearance, to start with: very round, relatively small and usually clustered into tightly packed colonies. A colony looks a bit like a pizza through the microscope. For conclusive proof, we test for marker proteins of pluripotency and have to show that the cells are capable of differentiation into the body’s various tissue types.

**And that’s where cardiac modeling comes in?**

**Moretti:** Indeed. Around ten days after adding a specific differentiation cocktail, the iPS cells form an embryoid body.

**An embryo?**

**Laugwitz:** No. A type of tissue that generates various cell types but is not a functional embryo.

**Moretti:** After another few days, you can see cells here and there in the tissue that are beating regularly – just like cardiac muscle cells. Those are the ones we want, and we use them to culture myocardial tissue. In total it would take us five months to reach that stage with your blood sample.

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**Ein neues Krankheitsmodell für die Herzforschung**


**Bahnbrechende Fortschritte**

In einem international hochkompetitiven Umfeld schaffte die TUM Arbeitsgruppe dabei gleich mehrere Premieren:

- 2010 erbrachte sie den weltweit ersten Nachweis, dass sich mit diesen Zellen genetisch bedingte Krankheitsursachen tatsächlich neu aufklären lassen. Im konkreten Fall die einer bestimmten Form von Herzrhythmusstörungen, einer Variante des sogenannten Long-QT-Syndroms.

- 2013 konnte bei einer zweiten Form von Long-QT der zugrunde liegende genetische Defekt molekulargenetisch behoben werden – noch nur in der Zellkultur.

- 2014 gelingt durch sogenanntes Exon-Skipping die Korrektur eines genetischen Defekts bei einer Form von Herzmuskelschwäche, bei der das Strukturprotein Titin defekt ist. Solch ein Exon-Skipping lässt sich womöglich bald auch klinisch erproben.
The cells have a pulse?
Laugwitz: Yes – some of them beat continually at the same rate as the human heart, so 60, 70 beats per minute. We can keep them in culture for up to a year.

Are you aiming to grow this kind of cardiac muscle tissue in the hope of surgically replacing damaged tissue in heart attack patients?
Laugwitz: No, we’re a long way from that. There are various challenges to overcome, including the immaturity of these cardiac muscle cells.
Moretti: What we’re currently obtaining are very young, immature myocytes – comparable with those of a newborn.

But, for the first time, this system gives us access to human heart muscle cells for research.

Wasn’t that possible before – in connection with organ transplants, for instance?
Laugwitz: Occasionally, yes. But adult cardiac myocytes survive just six hours in the lab. Only now, with iPS cells, we can produce cardiac myocytes for every genetic heart condition and potentially from every individual. In fact, back in 2010, we were the first in the world to generate such cells from cardiac patients.

What sort of patients were they?
Laugwitz: A father and son who both have long QT syndrome or LQTS.

Long ... what?
Laugwitz: The name comes from the prolonged QT interval visible on electrocardiograms or ECGs for these patients. In healthy individuals, the frequency-adjusted QT interval lasts a maximum of 440 milliseconds but in people with this condition it is longer: up to 700 milliseconds. The T wave of the ECG represents the repolarization phase of the heartbeat, when the cardiac muscle relaxes following contraction. Since this takes longer in these patients, it can cause abnormal heart rhythms (arrhythmia).
What symptoms do these patients have?
Laugwitz: Often hardly any at first – the condition frequently goes undetected in the early stages. Warning signs can include fainting or an unexpectedly rapid heartbeat (tachycardia). Congenital heart arrhythmia is like a time bomb, increasing the risk of sudden cardiac death – particularly when under strain as a result of stress or sports, for instance.

Is there any treatment?
Laugwitz: There is no causal therapy. We usually prescribe beta-blockers. In severe cases, we recommend preventive placement of an implantable cardioverter-defibrillator (ICD). Fortunately, the condition is very rare, affecting maybe one in ten thousand people. And it was from two of those patients that we were able to generate iPS cells and, subsequently, cardiac muscle cells in the lab for the first time in 2010, giving us a better understanding of the mechanism of the disease.

What did you find out?
Laugwitz: Well, we have known for a while that, in most long QT patients, certain ion channels in the heart cells no longer function properly. In the most common variant, LQT1 – also the type affecting these two patients – the functionality of specific channels for potassium ions is reduced. After the cell contracts, it is immediately flooded with positively charged ions. These KCNQ1 channels then open up to allow the positively charged potassium ions to flow back out of the cell, neutralizing the excess charge so the cell is ready for the next contraction.

Which, of course, takes longer if the channel doesn’t work properly. So you already know all about the disease? Then why the heart models?
Moretti: No, that’s not true – no one knew what was really going on with the diseased channels inside human heart muscle cells before.
Laugwitz: The great thing about iPS technology is that we can now for the first time examine in patient-specific cardiac muscle cells exactly where and how many of which channels are synthesized and how they behave. At the same time, we are able to measure electrical activity even at the level of a single channel. This puts us in the position to analyze the concrete implications of individual genetic defects on cardiac activity of the patient. In the end, we were able to identify a transport defect in both patients.

What sort of transport defect?
Moretti: The KCNQ1 channel is made up of four identical subunits within the cell, which then reaches the cell membrane. These patients have one normal and one mutated copy of the gene for the subunit and their cells combine...
“Before we could investigate the long QT syndrome using cardiac muscle cells produced from iPS cells, no one knew what was really going on with the diseased channels inside patients’ hearts.”

Alessandra Moretti

both forms during synthesis. What we have seen is that a channel with more than one defective subunit cannot reach the cell membrane. So the patients have less functioning channels in their membrane compared to a healthy person. In a nutshell, that also explains why the repolarization phase of the action potential in their cardiac muscle cells and thus the sum signal of all cells – the QT interval on the ECG – is prolonged. The potassium doesn’t leave the cells quickly enough because the channels to enable it are missing.

So do your investigations benefit the patients?

Laugwitz: Not directly, at this stage, but we are definitely a step closer. With these two patients, we now know that drugs could help resolve the transport issue and ensure that more of the defective channels reach the membrane, where they would then function sufficiently. But such pharmacological agents don’t exist yet – they still need to be developed by industry.

Couldn’t you glean this information just as well from animal models?

Laugwitz: Not at all. The mouse, in particular, has very little relevance here, since its heart physiology is completely different from ours. Mice don’t die from cardiac arrhythmia, even if they lack the ion channels that are essential for the human heart function. No – we are only able to explore the exact causes of these conditions in detail now that we have access to human heart cells. Long QT alone has 14 variants, with genetic defects in a wide variety of channels, so we have a lot more projects ahead of us.

And yet the value of these human heart models is controversially discussed even among experts. A dozen groups around the world are working, like you, with these iPS-derived models. Just last year, the Circulation Research journal published a heated exchange between you and a colleague, Björn C. Knollmann from Vanderbilt University in the US. According to Knollmann’s calculations there, a single heart model generated in this way would cost over 15,000 US dollars …

Laugwitz: It is expensive, yes.

… and he argues that, while the models might be helpful in investigating genetically determined arrhythmia, these conditions are so rare that the industry would not invest in developing drugs for such a small market.

Laugwitz: We take a different view. The cost of these models will decrease – industry itself will make it possible.

Why is that?

Laugwitz: A test for QT-interval prolongation is now mandatory for every novel drug compound before reaching the …
Induced pluripotent stem cells (iPS cells)

... were first created in the lab of the Japanese researchers Shinya Yamanaka and Kazutoshi Takahashi, who succeeded in using four specific proteins to generate pluripotent stem cells from mature, differentiated somatic cells in mice in 2006 and in humans in 2007. Pluripotency is the ability of a cell to form all of the roughly 220 differentiated cell types present in the organism. A counterpart of iPS cells are embryonic stem cells (ES cells), which can also be obtained from humans through somatic-cell nuclear transfer (SCNT) since 2013. Here, the nucleus of a differentiated, somatic cell is injected into an egg whose genetic material was previously removed. Once ES cells are harvested from the early-stage embryo produced in the lab, the latter is destroyed. ES cells are regarded as easier to cultivate and propagate than iPS cells. However, iPS cells raise fewer ethical issues, since their generation bypasses the need for early-stage embryos.

market and being used in the clinic. Indeed, many pharmacologically active substances carry the risk of prolonging the QT interval and, depending on the circumstances, might then not be authorized for human use. Right now, animal models are used for these tests, but human cardiac cell culture systems offer so many advantages that the pharmaceutical industry is making intensive efforts to establish human iPS-based models for drug development. Which then could also reduce the costs sufficiently to enable the use of personalized models to test whether a long QT patient would tolerate a specific drug. Even many authorized drugs, such as antibiotics, can increase the risk of arrhythmia in these patients. If someone with this condition needs antibiotics, there are risks that we are not yet able to evaluate properly.

But as you’ve explained, it takes almost half a year to develop such a model. So there surely wouldn’t be time to test the antibiotics in case of acute infection?

Moretti: We could generate for long QT patients prophylactic iPS-based heart models. But of course we also have a more ambitious aim – developing actual treatments. We are currently conducting a study showing the feasibility of this.

Can you tell us more?

Laugwitz: Well, more common than long QT are cardiac diseases known as dilated cardiomyopathies, in which the heart muscle is enlarged and weakened. In every second patient, mutations in the gene encoding the protein titin are responsible for this disease. Titin is a component of elastic structures – sarcomeres – in the cardiac muscle cells. If titin defects are present, these sarcomeres stretch out and the heart pumps with less force.

And how does your new treatment work?

Laugwitz: The gene for titin consists of numerous coding regions, called exons. In our patients, the genetic sequence is defective in just one exon. But as a result, protein synthesis in the cell is interrupted at this exon and no viable titin is produced. Through tests on iPS-generated heart models from these patients, we have now been able to show how we could prevent this synthesis stop on the part of the protein – by using exon skipping.

What is that?

Moretti: It’s a method that involves the smuggling of nucleic acid particles, or oligomers, into cells so that the protein synthesis mechanism simply skips over the faulty exon of the gene and completes the rest of the protein. This results in generation of largely functional titin within the cells. And, most importantly, the sarcomeres gain stability and cell contractility increases. Clinical trials with exon skipping are already ongoing for other conditions, particularly muscular dystrophies, and we also see potential for its use in cardiology.

And now a practical question to finish off with: do you plan your experiments together or does each of you do your own thing?

Laugwitz: Oh, definitely together.

Even at home over the dinner table, I suppose?

Moretti (laughs): I wouldn’t rule it out. But some of our best ideas come when we’re visiting my family in Padua and relaxing in the vineyards.

Interview by Bernhard Epping