The Case of the Short-fingered Musketeer

Chapter 21

by Russ Hodge

copyright 2015

Fred Luft

21 Sometimes, an answer

It's January 2015, and it's freezing in Berlin. A cold wind scours the MDC, slipping between the buildings and rattling the leafless branches in the campus woods. From the windows of Fred Luft's office you can still see tiny islands of snow, deposited just after Christmas, the last holdouts after a few warm days. But for now the sky is solid grey, and the air is cold enough to put dents in you. It's exactly the way you have always imagined Upper Norway.

Fred has his feet up on the corner of his desk, waiting, waiting. "We're paralyzed," he says. "Our paper has been at the journal for seven weeks now. Until we hear back, we can't do a thing."

Seven weeks without a response from an editor is not unusual, especially over the Christmas holidays. Still, the silence is ominous. So much depends on whether or not the paper is accepted. A rejection would be crushing. Almost as bad would be a request for more experiments that might be difficult, time-consuming, maybe even impossible.

Both things happened a year ago, after a first submission to the *New England Journal of Medicine* that was rejected. Fred sent the paper off again to *Nature Genetics*. Comments from expert reviewers, whose response determines the journal's decision, sent everyone back to the lab for more than a year of hard work.

Now the lab has done their best to satisfy the demands, but there's always the chance that someone will want even more evidence. And then there is no guarantee that the experiments will work out. In so many ways this is a do-or-die moment for the project that Fred's lab has now pursued for just over 20 years.

It has been two years since the events described in chapter 20, which closed the first edition of this book, and a lot has happened. In mid-2013, the group suddenly and rather unexpectedly discovered an answer to the question that sent Kemal off on a quest for help for his family over 50 years ago. The findings provided an explanation for the high blood pressure that affects a Turkish family living on the Black Sea Coast and others around the globe who suffer from Bilginturan's syndrome. Once again, participation from the family played a key role in its discovery.

But in the world of research, there is knowing and then there is *knowing*. An answer does not necessarily satisfy the stringent criteria for evidence required by the scientific community. The process is considered accomplished only when a piece of work has been submitted to a journal, has undergone a rigorous review by experts in the field in question, and then finally been accepted for publication.

Until then, knowledge remains carefully guarded in the lab. There is always the danger that another group will get a hint that allows them to apply new methods to a problem and win the race to publication. Or someone may make the same, crucial discovery while investigating a completely different problem. The longer things draw out, the more likely that such an event is to happen.

Getting scooped doesn't necessarily mean that the work will never be published, but it certainly reduces the chances that it will appear in a prestigious journal such as *Nature Genetics*. And that end, for better or worse, is crucial for a scientist's career. Other scientists use it as a measure of your talent and skills as a researcher – whether in prolonging your contract, promoting you in your present job, inviting you for a job interview elsewhere, considering your next paper, inviting you to give a talk at a conference, or evaluating your work for potential funding.

Besides such considerations, time is running out for Fred's lab. He has passed that "certain age," lost his clinical position at the Charité medical school due to mandatory retirement regulations, and is struggling to hold onto his group's budget at the Max Delbrück Center. His position has just been extended for another year, and that extension will probably be his last at the MDC. Without the publication, the project is likely to unravel very, very soon.

A publication in *Nature Genetics* would be a sign that all of that effort – and Fred's attitude on non-hypothesis-driven research – had delivered results that were important in the eyes of the scientific community. Not to mention that it would be a nice way to round off a project that had occupied his team for so many years.

We reach February, and one day at noon Fred finally gets fed up. He sends off an e-mail entitled "Friday the 13th," in caustic defiance of superstition, which contains only seven words:

"I thought today might be the day."

The only thing he adds is his name and the number that has been assigned to the paper by the editor. Then he leans back in his chair, laces his fingers together, and glowers at the monitor of his computer for a while.

By the end of the afternoon, he has a reply.

Chapter 20 left off as Fred's lab had decided to approach the Turkish family one more time and invite them again to Berlin. The idea was to try to obtain more cells from affected family members, as well as to carry out more detailed clinical studies. While the lab had found a mechanism called *CISTR-ACT* that might explain short

fingers in general, the causes of the brachydactyly in this particular family – and especially their extremely high blood pressure – were still unclear. The best clue to the causes of hypertension remained the unusually thick layer of smooth muscle that had been found around the blood vessels in affected patients.

There ought to be a genetic reason for this thickening. "There might be a contribution from a signal that causes the vascular smooth muscle cells to constrict," Fred said. "According to Ohm's law, all hypertension must involve an increase in total peripheral resistance."The effect might involve a change in some aspect of smooth muscle cells: their development, reproduction, or recruitment to the vessels they surrounded. But so far the scientists hadn't discovered a genetic basis for the difference. And there was no way to tell whether or not the concepts needed to describe it even existed – as yet.

For 20 years, the family's short fingers had beckoned the lab along, luring them forward, sometimes appearing close and within reach, only to slip away again as they felt they were getting close. "But the short fingers were an unexpected gold mine," Fred says. "Colleagues sent the lab samples from patients with type E brachydactyly. Hypertension is common – half of us get it. So samples were available from persons who did not have the same syndrome, but had the same fingers."

It was a diversion, he calls it, but it caused the lab to discover regulatory components such as gene enhancers, and *CISTR-ACT* in the development of short fingers. Nonetheless, the cause of the high blood pressure was still dancing away. At the same time, they were getting better at detecting it – during the 2009 trip, they found signs of hypertension even in young children. And with the discoveries about the landscape of chromosome 12, they felt they were getting closer.

It would be good to have more cells from those affected by the syndrome. After all, such cells had contributed to the *CISTR-ACT* project. A single sample obtained during the trip had allowed them to develop stem cells for use in experiments in the lab. More cells, from healthy and affected family members, might reveal cellular factors that contributed to the thickened vessel walls.

The group contacted the family to begin organizing another visit. Fred composed a letter, which Atakan Ayadin translated into Turkish, and they sent it off to Cafer.

And then things started to fall apart.

By that time it had been four years since our trip to Cafer's home in Turkey. Seeing the family again was an exciting prospect. Although we had spent only a week on the Black Sea Coast, I had vivid memories of many of the people we'd met. While writing a book, you develop a special relationship with its characters. Each drifts into the story in the midst their lives, briefly intersects with the other characters, and then continues – along a changed trajectory?

It would be interesting to see how things had turned out, particularly for the children. What had become of Mehmet's daughter Tülay, the poetess – had she married? Had Cafer's son Ercan pursued his interest in philosophy, or followed in his father's footsteps to study religion? Were the Hoca's bright and lively daughters at the university, studying to become teachers?

The first piece of news from Karamat, however, was saddening. For the first time in nearly two decades, Bilginturan's syndrome had claimed another life, and not that of someone you might expect – an older family member who had lived with the condition for a long time. Instead, once again, the disease had taken someone in the prime of his life, a young father not yet 30 years old – someone we had met and who had been extensively studied during various trips to Turkey or Berlin.

Sylvia Bähring, Jens Tank, Okan Toka

It was a direct and tragic reminder of the real and devastating consequences of the syndrome, and the way it had interrupted the lives of a family for over a century. The intervention of Fred's group had changed things a lot; during his year in Turkey, Okan had found drugs that could lower their blood pressure, and then the Toka brothers and Atakan had made regular trips to deliver them. Those efforts had made the ultimate consequences of the disease more abstract, maybe even a bit unreal. Being told that a genetic condition might kill you is a lot different than watching relative after relative die.

The young affected person didn't have the experience of his older relatives, who recalled a time when the syndrome regularly plucked people from their midst. For them, the threat was always there, lurking in the background.

But like so many of the young people of Karamat, the young man had moved away from his native region in search of a job. In doing so it was surely easy to become somewhat disconnected from the stories and traditions of the family. During our visit he had mentioned that the drugs he had taken to lower his blood pressure made him feel listless and sluggish. Eventually he stopped taking the medications that could have saved his life, and the syndrome claimed its next victim.

The letter to Cafer provoked a disappointing response: no one from Karamat would make the trip. We could speculate about the reasons – had the syndrome also receded into the background for the rest of them? Was it worth making the effort when it never seemed to lead to any practical benefits? Speculation was useless; his decision was firm, and we'd never change his mind over the phone.

But there were other members of the family who weren't subject to Cafer's decision. Atakan contacted a small branch of the family that had moved away from Karamat a few generations ago and settled in another village near the Black Sea Coast. He also got in touch with the family that had moved to Stuttgart.

Both groups were willing to make the trip to Berlin, and the lab began making preparations for their visit. Organizationally, it would be harder this time because of Fred's having been "retired" from his position at the clinic. The group would have to arrange everything themselves, both before the visit and while the family was in Berlin.

Over the many years of this project, Fred's group has continually had to learn new methods to ask the scientific questions they wanted to pursue. Now a parallel situation was happening in terms of organization and administration. The scientists had never had to organize and coordinate – on their own – a visit and a number of clinical trials.

"We had to work as a team in a new kind of way," Sylvia says. "Each of us would have multiple responsibilities during the visit. First we had to get the family to Berlin. When they arrived, we would need to get them settled and take care of them, arrange a sensible schedule for all the tests that we wanted to perform, and accompany them from place to place."

Alongside all of these other activities, the group would be performing tests and carrying out procedures that in many cases they hadn't done. Sylvia says. "That meant we had to practice on control subjects – and each other."

A main goal of the visit for the scientists would be to obtain specific cells called *mesenchymal stem cells* (MSCs) from affected family members and their healthy relatives. In the lab, these cells can be stimulated to develop into *chondrocytes* – cartilage cells that play a key role in bone development.

During the visit in 2009 the scientists had attempted to obtain MSCs from patient blood samples and had failed – with the single exception. But the cells were easier to

extract from abdominal fat, so this time the lab would perform fat biopsies to extract MSCs, using a procedure common in "aesthetic" surgeries.

MSCs could also be transformed into smooth muscle, which was the current aim. If the family's hypertension was really due to differences in the amounts of smooth muscle surrounding their blood vessels, the scientists would have to show that these cells behaved differently in affected family members. They would also need samples from their healthy relatives as controls.

The team also wanted to take a much closer look at the family's heart functions. So far there had been no indication of the types of organ damage that normally accompanied hypertension. Usually a person who experiences a significant rise in blood pressure, over the long term, exhibits cardiac *hypertrophy* – like any other muscle that works hard, it grows in size. The way to find out was through echocardiography. Fred and his colleagues had already used the method to look at the family's hearts back in 1996 and hadn't found anything unusual about the organ. They didn't see any enlargement of the left ventricle, which actively pumps blood into the vascular system. This suggested that whatever was raising blood pressure was accompanied by some sort of protective effect.

But there had been major improvements in magnetic resonance imaging technology since that time. Now you could watch the heart as it pumped and catch a detailed view of its substructures in action. It was worth taking a second look, to see if something had been missed the first time around. Jeanette Schulz-Menger, head of Cardiac Magnetic Resonance Tomography at the HELIOS clinic across from the campus in Berlin-Buch, would be brought in to help.

Jens Tank, a former member of Fred's lab who was now at the Medical School in Hannover would organize these clinical studies. Jens had played an important role in the clinical work the last time the family had been brought to Berlin. In January 2013 he returned to have discussions with Fred and the group on the different types of examinations that might be carried out and to start coordinating that aspect of the visit.

One new test that would be performed would be to look at the activity of enzymes involved in the functions of *platelets*, fragments of blood cells that have a central role in clotting. Cause of death without treatment was stroke in the affected family members. The reason could be that platelets were more active in affected family members. Jens contacted a former colleague named Thomas Müller, a specialist in coagulation who now worked at a blood bank in the city of Oldenburg, and brought him on board to perform the coagulation studies.

In addition to the new procedures, the normal battery of tests would be carried out: measuring height, blood pressure, and taking blood samples. At least one new affected family member would be arriving. The genome of each additional subject would be unique. With a bit of luck, there would be changes in the region of chromosome 12 that the scientists had been investigating for so many years.

In 2012 the group had already obtained DNA from a member of the Turkish family who was being studied for the first time. What they discovered would be pivotal in understanding the hypertension associated with the disease.

In 2012 Sylvia and Atakan took the train to the city of Erlangen in central Germany to visit Okan Toka, the young physician of Turkish heritage who had spent a year in Karamat. There his extensive work had demonstrated that various combinations of anti-hypertensive drugs could lower family members' blood pressure to levels that would allow them to escape strokes and other deadly consequences of Bilginturan's syndrome.

Alongside his full-time position as a physician at the Children's Hospital of the University of Erlangen, in the Department of Pediatric Cardiology, Okan was doing his best to continue his research. But it was getting harder and harder. The privatization of the German hospital system of a few years ago had shifted hospital activity almost entirely toward patient care. Insurance companies would compensate those expenses; research, on the other hand, didn't bring in clear or immediate profits. Okan is still in Erlangen, although each year things have become a bit harder for him. "The German medical academic rat race is another story entirely," Fred remarks. Nonetheless, Okan continues to fight the good fight.

In Erlangen the three scientists were to meet a 16-year-old boy from the branch of family living in Stuttgart, which was much closer to Erlangen than Berlin. The father, who did not have the syndrome, accompanied the boy. The boy's mother was affected, which meant that her son had a 50 percent chance of having inherited the syndrome. The researchers hadn't ever examined the boy, or even met him – their last encounter with the Stuttgart family had preceded his birth. "I think his mother was pregnant with him at the time," Sylvia says.

So far everyone around the globe with the disease, all six families, met four criteria that could be determined without invasive medical procedures: short fingers, an overall short stature, high blood pressure, and a disturbance of the crucial region of chromosome 12. All of them exhibited a fifth symptom: a blood vessel in the brainstem that made an unusual loop around a brain-stem structure. The sixth criterion – a thicker layer of smooth muscle around the vasculature – required a biopsy.

The boy would be spared that procedure, but otherwise the plan was to X-ray his hands, measure his height and blood pressure, do an MRI to look for the looped brain artery, and take a blood sample. That would be taken back to Berlin, where DNA would be extracted and they would test the genetic markers for this small branch of the family, helping to resolve questions about the chromosome.

Even without this information, Sylvia expected they would probably have a solid diagnosis by the end of the day. In fact, if the boy were affected, she might see it right away. At the very least you would see the signs of hypertension, which could now be detected at an early age. By the age of 16, the boy's blood pressure ought to have risen significantly.

Sylvia's first impression when the boy came in was that he probably wasn't affected. "He wasn't tall, but neither was his father." Okan had him mount a scale: 159 centimeters. That put the boy's height, for his age, in the 30th percentile of his peers – comfortably within normal range. Compare that to a strongly affected cousin, who was 142 centimeters tall at the age of 15, putting him in the lowest *one* percent for children his age.

The boy's fingers might be a *bit* short – especially the little finger on each hand – but certainly didn't exhibit the extremely truncated form found in some of his relatives. A technician took X-rays, and Okan pulled the images up on his computer screen. The three scientists, the boy and his father gathered around to inspect the results. Okan gave a quick tour of hand anatomy, using a pen to indicate the various bones, starting at the tips of the fingers and moving toward the palm.

The first three bones, the *phalanges*, were often close to normal length in the syndrome. But they butted up against the next phalanges in cone-shaped ends, forming structures called *epiphyses.* In unaffected family members the ends were much flatter. The *metacarpals*, the bones that connected phalanges to the wrist, were usually much shorter in people with the syndrome. Both features have characterized the hands of every affected family member – so far.

The 16 year-old's fingers looked fairly normal. Well… mostly. The metacarpals of the thumb and first three

fingers didn't seem very unusual – they were certainly much longer than those of the boy's strongly affected, same-aged cousin and all the other affected relatives. The little finger, however, looked like a classic case of the syndrome. Fred "took the liberty" of showing the X-rays to independent pediatric radiologists at Vanderbilt University in the US. "No doubt about it," was the answer.

Fred remembers seeing the images later and shaking his head. "What were we supposed to make of *that*?" he says. "We had looked at lots of hands over the years, and if the person was old enough, it was pretty easy to tell whether they had the condition or not. One thing that had interested us from the beginning was that you could usually identify the syndrome just by looking at their hands. The combination of this with hypertension was what had attracted the attention of Nihat Bilginturan, and finally our attention – in the first place."

Okan measured the boy's blood pressure, which yielded a systolic value of 145, diastolic at 77. "That was clear," Fred says. "If you're sixty years old, a value like that would prompt your physician to tell you to start changing your lifestyle, and possibly to consider putting you on medications. If you're sixteen, it means there is something wrong."

In this case, the problem was Bilginturan's syndrome – but why didn't the boy have the other classic symptom: the short fingers found in every other person who had it?

"Suddenly we thought we might be facing the first case we'd ever found of *incomplete penetrance* of the syndrome," Sylvia says. She explains: many other hereditary diseases have symptoms that affect patients to different degrees. In other words, people with the same defect in their DNA might have extremely short fingers, while others might be closer to the normal range. In many cases the symptoms lie on a spectrum that makes it difficult to distinguish true cases of the syndrome from normal variation. That hadn't been the case for Bilginturan's syndrome: so far, everyone examined had extreme forms of both symptoms.

Left: an X-ray of a hand of a boy with the full syndrome.
Right: a hand of the 16-year-old from Stuttgart.

And another thing seemed to be going on, perhaps, that tremendously excited the group. Again, all the patients so far had displayed *both* major problems of the disease. That connection was so strong it suggested that a single gene or sequence of DNA triggered both problems.

Another possibility was that the syndrome was caused by two sequences lying nearly side-by-side. They might be so close that scientists had not yet found a person in whom the natural process of chromosomal recombination had split them. At some point, however, if you studied enough people with the syndrome, you would expect that to happen. This young man might represent the first known case of Bilginturan's syndrome where the phenotype of high blood pressure had been separated from that of the severe skeletal features. If so, it would be an enormously important discovery.

Everything depended on the critical region of chromosome 12. Would the boy's DNA reveal unique differ-

ences? Sylvia and Atakan jumped on the train to take the blood sample home. In terms of understanding genetic causes of hypertension, the sample might hold extremely important clues.

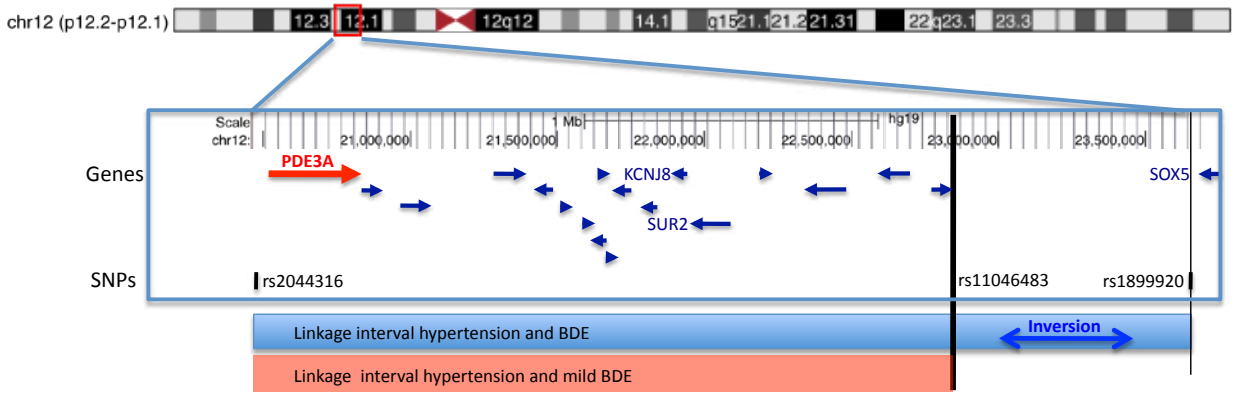
The analysis began right about the time I was finishing the first version of this book, as the group was starting to plan the family's visit to Berlin. I'd gotten vague hints that something was up in the lab, but they weren't ready to publish or talk about what they found. They didn't tell me much about their trip to examine the 16 year-old. While hypertension was a central theme of the story, there was no answer by the close of *The Case of the Short-fingered Musketeer*. Someday the time for this new chapter would come, but first there was a lot to do.

The sample from the boy in Stuttgart was now back in Berlin and the scientists got their first look at his DNA. The sequence confirmed what they had been hoping.

Half of his genome had been produced long ago in his mother's body, as pairs of her chromosomes were split up to produce an egg cell that would contain half of his future genome. During that process, a fragment of DNA broke off one of her copies of chromosome 12. In attempt to repair the damage, her cells had reinserted the sequence in what appeared to be the right place – but they placed it in the other copy of the chromosome. This moved a bit of healthy DNA into the region associated with Bilginturan's syndrome. It replaced only a part of the sequence that caused the disease.

This natural process of shuffling DNA between chromosomes is frequent and random. Scientists call it "crossing-over,," or a recombination event. By identifying the borders on either side of sequences that have moved, scientists close in on specific regions of DNA linked to genetic syndromes. Such events give linkage studies their power, because a particular DNA sequence must be inherited with the disease. Aligning and comparing different borderlines within the family and to those of other people suffering from the disease is what had led them to this region of chromosome 12 in the first place.

The most interesting thing was that the new sequence eliminated particularly the part of the region that had



Chromosome 12 (top), with a close-up view of the region associated with Bilginturan's syndrome (red box and bottom). The boy from Stuttgart, who has somewhat longer fingers but the full hypertension phenotype, inherited the syndrome through the region marked "Linkage interval hypertension and mild BDE," but a healthy form of the region marked "inversion".

occupied the lab's attention for a long time. In 2004, after years of work, Sylvia and her colleagues had shown that a part of the sequence had undergone a major rearrangement in everyone suffering from the disease. In everyone who was affected, the entire region of DNA had been re-inserted in the opposite direction. In some, the order of the sequence had undergone additional scrambling. But so far, an inversion had been found in every patient – including the boy's mother.

But in the boy, the inverted region had been replaced by the sequence from the mother's healthy chromosome 12. Yet he still had high blood pressure. This was a huge breakthrough – it meant that the inversion wasn't responsible for hypertension. Nor was it entirely responsible for brachydachtyly: the boy had a very mild form of it. The new information sharply narrowed the region that had to be searched for the cause.

"The effects on the boy's fingers were much less pronounced," Sylvia says. This means that the region with the inverted sequence influences the strength and regulation of the growth processes that affect bones of the fingers and skeleton. At the same time, the problem with hypertension is outside that part of the sequence, in a smaller part of the locus."

But where could the defect lie? Years ago, the group had wondered whether the hypertension and/or brachydachtyly might be due to a mutation – the alteration of a single letter of the family's genetic code, probably within the region of chromosome 12 associated with the disease. At the time, the lab had searched for such a change but failed to find one. Then, the discovery of the chromosomal rearrangement within the region had sent the group's work off in another direction.

"That direction was not fruitless," Fred says. "It led to investigations that culminated in *CISTR-ACT*. This had nothing to do with hypertension but was nonetheless good science. This underscores the notion that 'wild-goose' chases can nonetheless result in a good dinner."

The past decade or so had seen enormous advances in DNA sequencing technologies, introducing more automation and reducing the time and cost involved in obtaining a complete genomic sequence. A "thousand-genome project" was underway to sequence the genomes of individuals from across the world and compare them to the "reference" human sequence that had been published ten years earlier.

The result of this project and many others has been a very "high-resolution" view of the landscape of variation within our species. An individual's sequence deviates from the reference genome in many ways. It is full of single changes in the genetic code, called *single nucleotide polymorphisms*, or SNPs, which represent mutations that have occurred in an individual or one of his ancestors. It also contains insertions and deletions of sequences of various lengths. In some cases they were inverted, as the lab had found in chromosome 12, had been recombined, or undergone other types of changes. It will likely be many more years before scientists grasp the full extent of variation, and even more before they understand the influence of an individual's personal sequence – which is unique and entirely new – on his life.

The extent of individual genomic change is lower when the sequences of relatives are compared, because of the way sequences are passed from parents to their children, but it is still quite extensive. And the search for a single, critical mutation is also complicated by technological limitations that arise from the methods by which the information is obtained. Sequencing relies on enzymes that chew DNA into tiny fragments of different sizes.

When the order of the nucleotide subunits in this fragment is ascertained, the data has to be "assembled" – meaning to be reintegrate each sequence into its proper position in the whole. Its proper position is determined

by comparing the ends and finding matches within other partial sequences. This operation requires a very intensive computational analysis of huge amounts of data.

Atakan was looking for one letter of the genetic code that had to meet certain requirements. First, it would be found in every patient but none of the nonaffected relatives. Secondly, it would only occur on one of the person's two copies of chromosome 12. Third, the change would match a single substitution also found in one of his parents. Since no two affected family members had ever married, there had never been a case in which a person had inherited two copies of the defect, one from each parent.

Finding a mutation that met these criteria would require sequencing DNA from affected family members and at least one healthy relative. The data would have to be accurately assembled in a way that wasn't fooled by the fact that some regions might be scrambled, repeated, or inverted. Any of these larger changes might lead the computer to place sequences in the wrong locations, making it impossible to "align" them with each other and identify a single change.

The group had acquired some funding that they used to hire a company called Complete Genomics to sequence the genomes of three affected individuals and a healthy relative. The "deep sequencing" method that was used ran through the same DNA fragments over and over and over to ensure that the entire sequence would be captured and properly assembled. This produced a massive amount of data that takes up half of a four-terrabyte hard disk on the desk in Atakan's office.

The human genome is about three billion nucleotides in length. By factoring in the new border from the 16-year-old boy from Stuttgart, they had restricted the area to be covered to a thousandth of that total sequence. Still, three million bases remained to be investigated.

"We had to compare each single letter in that region, on two chromosomes of four people," Atakan says. "Even though the subjects were all from the same family, this produced about 10,000 differences in those eight sequences."

Atakan, Philipp, and Sylvia began analyzing the sequencing results. Then one day Atakan announced that he'd found it. Only one single letter of the genetic code met all the criteria.

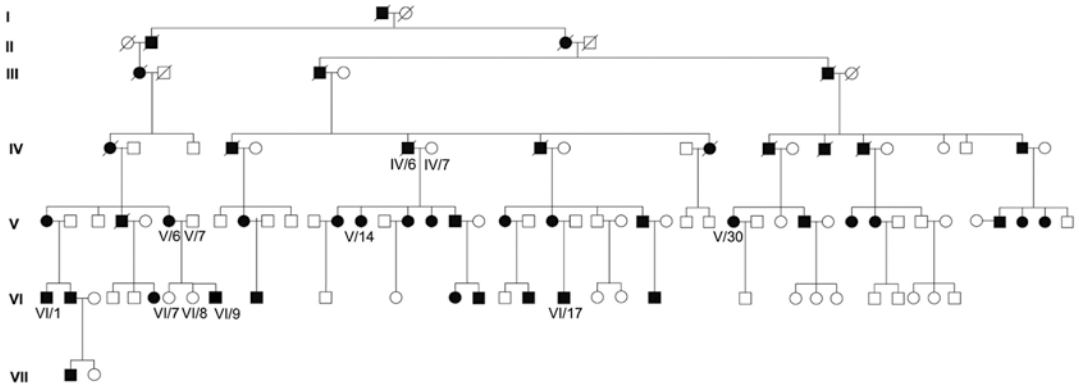
The mutation lay in a gene called *PDE3A*, which was located in the linkage region of the disease on chromosome 12. They had studied this gene before – but they had missed the mutation.

"How could we have missed it?" Fred says. "First, we'd sequenced only one family – the Turkish family. Resources are, after all, limited. And the methods are not foolproof. Conventional sequencing methods result in an error about every 1000 base pairs. If you have 3 billion base pairs, you can estimate how many errors can be made across a genome.

"If we'd found the mutation ten years ago," Fred says, "we probably would have had to let things go at that. At the time we didn't have the methods or tools to ask the next important questions."

Proving that once again, it was sometimes better to be lucky than to be right. The long years of waiting and frustration had allowed science to catch up to the project. The discovery was absolutely essential in answering the question that Fred Luft, Thomas Wienker and their colleagues had posed 20 years ago. Nihat Bilganturan had posed the same question 20 years before that – and he was still alive to hear the good news.

Two crucial questions arose from the discovery of the mutation. Did everyone else in the Turkish family who suffered from Bilginturan's syndrome, and was the mutation present in the families from France, USA, Canada, and South Africa? And how did the alteration of



The pedigree of the Turkish family. Roman numerals on the left: generations of the family. Black = affected individuals; white = non-affected.

a single chemical letter in DNA change cells and generate hypertension?

Answering the first question involved more sequencing. Fred's lab still had samples from the families they had found in France, the USA, Canada and South Africa. Now that they had a specific gene in mind, they could start by zooming in on *PDE3A*.

In the DNA from France and the USA, the scientists discovered different mutations in the *PDE3A* gene, but they all affected precisely the same amino acid in the protein produced by the gene. Healthy family members exhibited the normal sequence at this position.

The South African patient and one Canadian family also exhibited a mutation in PDE3A, but they found it at another position in the sequence – two amino acids farther down the protein. And in a second Canadian family PDE3A was also altered, at a position two amino acids past that. The three changes were so close to each other that they were almost sure to have very similar effects on the protein's functions.

In total, the project examined 48 subjects who had the syndrome. In every case they found mutations in one of the three positions. Interestingly, the protein has very close relatives in species across the evolutionary spectrum, and these positions in all of the molecules are occupied by the same nucleotide. This strongly indicates that the protein encoded in the *PDE3A* gene has functions that are essential to life, and that any changes may cause severe problems for an organism.

Single mutations often cause disease by causing certain types of cells to create a defective version of a protein, with a slightly different chemical recipe that alters the way it binds to other molecules. Other mutations prevent cells from making the protein at all. Investigating changes in PDE3A would require studying its activity in a cell – but what type of cell?

The most logical candidate was the smooth muscle that surrounded blood vessels. Getting such cells would require obtaining more mesenchymal stem cells from affected family members and transforming them into smooth muscle in cell cultures. The group would also need to study cells from healthy relatives and other people without the mutation, as controls. It was time for the family to return to Berlin.

Atakan had been working on the many formalities involving the travel of Turkish citizens to Germany. They had to have up-to-date passports and obtain visas.

He spoke to the authorities, wrote letters that had to be translated into Turkish, or from Turkish into German, and spent hours on the phone. He made an Excel table to help keep track of everything that needed to be done. Then it was a matter of ticking off the items on the list, one by one.

Someone from the family would have to visit the German consulate in Ankara. One of the men collected all their paperwork, got the travelers to sign a power of attorney, and set off on a long bus ride to the capital. The paperwork was completed just on time, the day the family's plane tickets arrived. Yvette Wefeld-Neuenfeld, a technician and mother with two children to take care of, had donned a third cap to play travel agent. She was put in charge of arranging the flights and getting tickets from the Turkish airline.

There were other problems; some who wanted to come couldn't get vacation time from their jobs. Two of the young people would have to interrupt their studies at the university. And what about health insurance – would normal travel insurance cover things if someone got sick? As a practicing physician, Okan could look into that.

Astrid Mühl worked with the administration of the campus in Berlin-Buch to arrange rooms in the guesthouse. Jens Tank had already made a schedule for the various tests with the HELIOS clinic and the Experimental and Clinical Research Center, which Fred had established on the campus.

Finally everything was ready. On a Saturday in October, 2013, one group climbed into the train to ride up from Stuttgart; the other boarded a flight in Trabzon and switched planes in Istanbul for the flight to Berlin. When they arrived at Tegel airport, Atakan and Okan were waiting.

There they had to deal with the first snag: one of the Turkish men didn't have the right stamp in his passport. They got that settled after a discussion with a customs official. While this was going on, several members of the group were bringing in groceries and setting up an impressive spread of food in the guesthouse, delivered by a Turkish caterer. And others were off getting things ready for the experiments they would perform.

Finally the group began trickling in. Ifaket, a cheerful, woman from Turkey who was affected by the disease, introduced her tall, lean husband as Atakan translated. We had met them both during our trip in 2009. She also presented two younger relatives, who had relocated to attend the university in the city of Bursa, south of Istanbul.

One of them was a young woman named Berrin, who was reluctant to say much; her brother answered most of my questions . He was taking his duties as chaperone – and possibly bodyguard – quite seriously. He never left her side and obviously intended to filter every question that was asked of her. The syndrome affected Berrin. Her blood pressure was clearly high when they measured it the next day, but she was young and the symptoms probably hadn't yet caused any significant health problems. Several times she insisted that she was fine. It's hard to be told that your genes aren't acting in your best interest, and many people's response is to deny that there is a problem at all.

This was an immediate reminder of the obstacles we had faced in 2009 – linguistic, cultural, and scientific. Such barriers are often sharpened by transplantation to a very strange, very foreign environment. When accompanied by your landsmen, old habits are often the best way of finding a comfort zone.

Most – but not all – of these difficulties were greatly reduced in our dealings with the family from Stuttgart, all of whom spoke fluent German. The group included a robust man of about retirement age who had lived in Germany for decades; his sister, who had come over a few years later and was affected by the disease. Also along were two adult daughters, one of whom had a small child

Family members from Stuttgart and Turkey with the group at a Turkish restaurant in Berlin

along that crawled around the dining room at the guesthouse and tried to open the cabinets. He was passed from relative to relative.

Their arrival was a joyous reunion of the older relatives, some of whom had not seen each other in many years. They ate, drank, and traded stories. We gave them copies of this book and they leafed through it, chattering about the people and places they recognized in the pictures.

The next day everyone gathered for a long breakfast at a restaurant on campus, where long tables had been arranged to fit us all in. Right afterwards they would all be whisked off to various rooms in the clinics and start the more stressful part of the week, but at the moment things remained fairly relaxed.

Okan stood up, gave a short overview of what they should expect over the next few days, and distributed a two-page handout that explained what would be done and why. The procedures were routine, he explained, and might be a little uncomfortable, but that would be brief and at the end of the week they'd have a chance to explore the city.

The visit achieved everything the scientists hoped, providing data and material that would serve as the foundation for their further work on hypertension. While the main goal was to obtain mesenchymal stem cells to be used in studies of smooth muscle, other types of cells produce PDE3A. The protein is also found in the heart and in cells called *thrombocytes*, or platelets. Experiments would try to determine if mutant PDE3A affected their functions in blood clotting,

Jens Tank had organized some work on platelets that would be carried out by Thomas Müller and some of his colleagues, Andrea Doescher and Simone Gnoth. They drove up from Oldenburg with a truckload of their own equipment and computers – yet another example of how the project kept drawing in enthusiastic participants. The results of their work have not yet been published.

High-resolution MRIs and echocardiography, carried out by Wolfgang Utz and Annieszka Töpper, gave the scientists their closest view yet of the families' hearts. The imaging revealed that their condition had not caused hypertrophy of the left ventricle, an enlargement that often accompanies long-term hypertension.

The best way to obtain mesenchymal stem cells was probably from abdominal fat, which required a small operation to remove bits of tissue. Knut Mai and Gabriele Rahn in the Clinical Research Unit of the ECRC performed the procedure. The biopsies weren't dangerous, or painful, or difficult to perform – about as uncomfortable as drawing blood. The tissue from the biopsies had to be carefully handled and was delivered straight into the hands of Yvette, Irene Hollfinger, and Astrid Mühl. They worked with Philipp to extract MSCs, removing other types of more differentiated cells. The transformation into smooth muscle required several weeks of culturing and stimulation with specific factors, a process that would take place in the weeks following the visit.

The fact that the scientists not only had to help in the clinical work, perform their own experiments, and shuttle patients from station to station meant that by the end of the week, they were totally exhausted. After the family had left, they met over a beer to decompress.

"Without any experience in all of this, it was pretty amazing everything worked," Sylvia says. "All of this could have created enormous stress and tension, but instead we worked together as a great team. That was a real highlight for everybody."

The mesenchymal stem cells could be used in combination with various experiments to explore the

Gabriele Rahn and Knut Mai taking performing a biopsy on a non-affected family member

functions of PDE3A, the molecule that had undergone mutations in every person with Bilginturan's syndrome.

Scientists already knew several things about the molecule that would be helpful in understanding its functions. PDE3A belongs to a family of closely related molecules that were already known to be involved in finger development, but how did the mutation affect smooth muscle?

PDE3A was known to have an effect on biochemical signals that cells use to control important processes such as growth and replication. It indirectly affects whether certain signals from outside the cell are passed to the interior. Such signals often rely on two small molecules called *cAMP* or *cGMP*. These molecules are switched on in response to signals from the cell exterior. Then they become transmitters, like a sort of "PR molecule" that widely broadcasts the signal to the cell interior. PDE3A helps control such signals by coming around once in a while and doing the equivalent of slicing off their antennas. It cleaves off segments, which reduces the molecules to forms called AMP and GMP.

Mutations might affect *PDE3A* in different ways. They could make the protein more active, which would reduce amounts of cAMP and cGMP and thus produce more AMP and GMP. Or the mutations could generally lower PDE3A activity, leaving levels of cAMP and cGMP unusually high. Then the fact that PDE3A cleaves both types of molecule suggests a third possible effect: it might increase PDE3A's preference for one molecule over the other.

One way to find out would be to introduce the mutant form of PDE3A into a cell and watch what happened to levels of cAMP and cGMP. Then you would repeat the same experiment with healthy PDE3A. If mutations were changing the protein's activity, you'd see it by comparing the amounts of cAMP and cGMP that were left over at the end.

When Philipp, Irene and Carolin Schächterle did this experiment, they found that the Turkish mutation was increasing PDE3A's appetite for cAMP. cAMP was binding to this target enzyme at a higher rate, producing more AMP, and leaving less cAMP available for other things than in people with a healthy form of PDE3A. On the other hand, the mutation didn't seem to be affecting the way PDE3A handled cGMP at all. So the mutation was probably blocking a lot of signals that would normally be transmitted by cAMP. Those passed along by cGMP, on the other hand, were getting through.

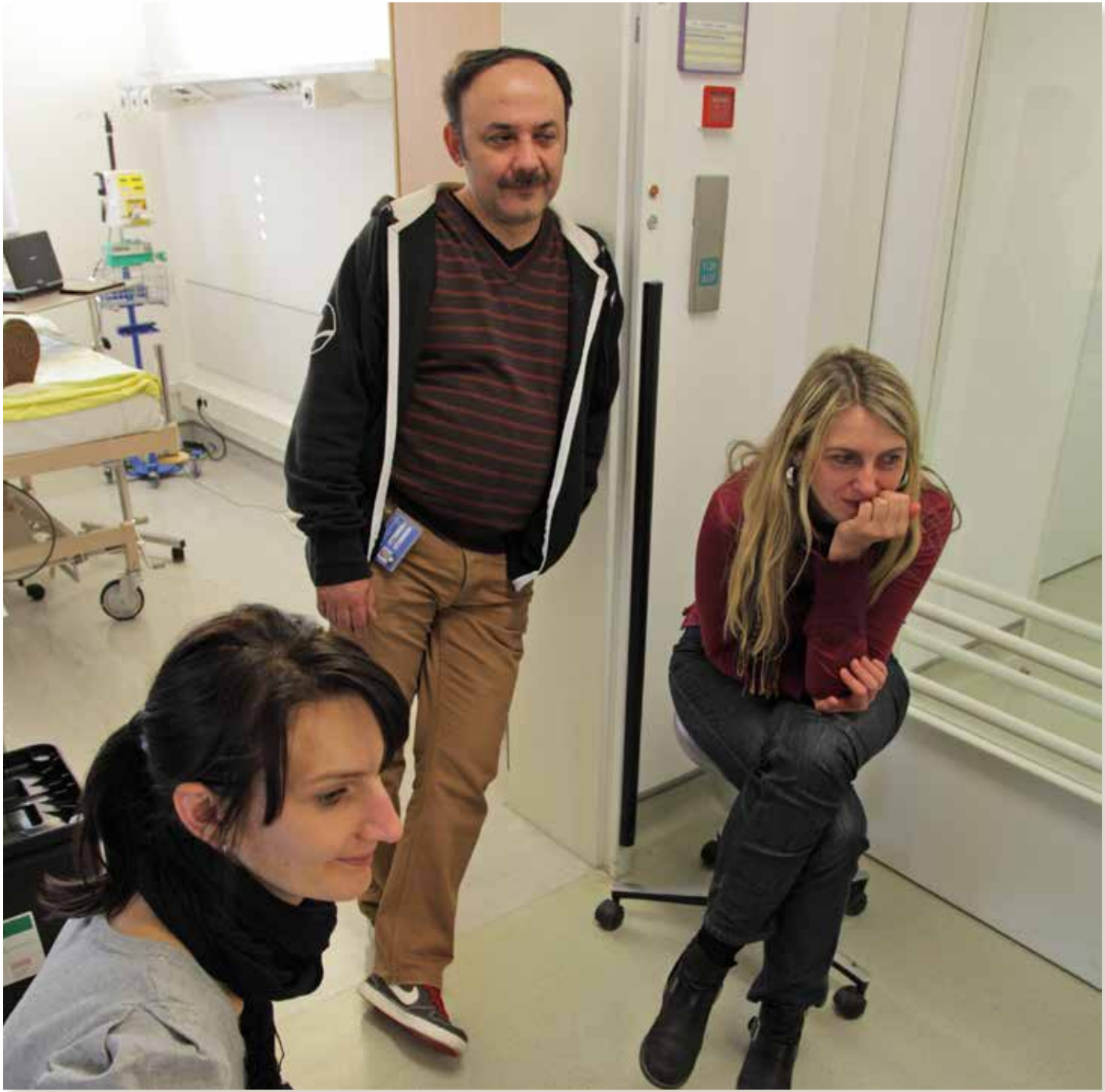
Ultimately, Philipp tells me, the increased breakdown of cAMP by PDE3A might help explain the family's short fingers. And it could do so by involving another nearby gene on chromosome 12, *PTHLH*, which he had studied intensively during his work on CISTR-ACT. Studies of mice had shown that their bones need PTHLH protein; without it, there are skeletal defects.

I asked him about the connection between *PTHLH* and *PDE3A*, besides their proximity on chromosome 12?

"Signals passed through cAMP stimulate the production of *PTHLH*," Philipp says. "So what happens if there is less cAMP? You'd get lower amounts of the protein. So when might you expect to find less cAMP?" He waits for an answer, looking at me with raised eyebrows. Oh, I suddenly realize; this is a test.

"In cells with a mutant form of *PDE3A* that was breaking it down?" I say, tentatively. He smiles – I've passed. For now.

No one in Fred's team had much experience with cAMP signaling, but another lab at the MDC did. "It was a fortunate state of affairs, but you shouldn't consider it an accident," Fred says. "While the MDC is mainly a basic science institute, it is charged with fostering collaborations between fundamental researchers and clinical scientists. Here's a case where the principle worked."



Yvette Wefeld-Neuenfeld, Atakan Aydin, Sylvia Bähring

Enno Klussmann's group had plenty of experience with cAMP; they had even worked specifically on PDEs, which meant they had the methods you'd need to study *PDE3A* mutants and their effects on cAMP signaling.

Enno got a call from Fred's group toward the end of 2013. "I think they had just tried to submit a paper on PDE3A to the *New England Journal of Medicine*, and it had been rejected," he says. "They had been trying to do something with cAMP signaling, and maybe they needed more experimental work."

Did Enno have time to meet? "Sure," he said, and invited them over for what he expected to be an informal chat. "The next thing I knew, there were four or five of them at the door." Enno had collaborated for years with Mathew Movsesian: "Matty", Fred calls him – an Armenian from Utah who had great expertise in phosphodiesterases and who joined the group as well.

Enno was brought in on the project along with Carolin Schächterle, a redhead from Munich, who had recently completed a PhD in biochemistry. She had just joined Enno's group as a postdoc, and her very first project would be to carry out measurements of cAMP in cells, collaborating with Philipp, Atakan and Sylvia.

"I really liked discussions with Phil that went on for hours and hours, as we tried to optimize the tests we were doing to make the measurements," she says. "We complained a lot, were frustrated a lot, drank some raki – and then suddenly we started to get the results we were hoping for. It was great to join this really interesting team – and to have the first project turn out successfully."

Another aim was to show how very precise alterations in the structure of PDE3A increased its cAMP-processing activity. Each of the three mutations introduced a slight change in chemistry in the same small area. It was an important site in the molecule. Other proteins could come along and glue *phosphate groups* onto it – which energized the molecule. That increased the amount of cAMP it would process. The chemical changes of the mutants made it easier to tag PDE3A with phosphate groups, and it went on to process more cAMP.

Yvette had been working with the smooth muscle cells raised from the patients' mesenchymal stem cells. She was following up on findings made in 2011 by scientists at the National Institutes of Health in the US. They had found an important connection between PDE3A and the behavior of smooth muscle cells: they stopped dividing in mice that didn't have PDE3A.

Here the situation was the opposite: her cells not only had PDE3A, but they had a mutant, hyperactive form. Maybe that would cause more rapid cell division. To find out you'd have to watch – and count – the rate at which smooth muscle cells divided. Yvette spent a lot of days in 2013 doing just that. When she thought she had enough data she brought it in and pored over it with Atakan and Philipp.

Their evaluation showed that it took about 36 hours for "normal" cells to double their number. But cells with the mutant form of PDE3A doubled about six hours faster. If this trend continued, at some point you'd see a significantly thicker layer of smooth muscle around blood vessels in the patients. Knowing that mutant PDE3A triggered faster growth didn't explain why it was happening. But the situation made sense if you assumed that cAMP was braking growth, and the mutant proteins really liked to release the brake.

Fred can think of several mechanisms that probably connect cAMP to hypertension via smooth muscle cells. "One is that a buildup of cells will increase the resistance of blood vessels, to the point that they can no longer flex under higher pressures," he says. "And remember that cAMP has an important role in muscle activity. When your muscles contract, proteins are getting cinched together. It

Yvette Wefeld-Neuenfeld, Eireen Bartels-Klein, Irene Hollfinger, Astrid Mühl

Enno Klussmann

Carolin Schächterle

takes energy to loosen them, and the whole process is dependent on cAMP signaling."

And any system involving cAMP, he says, can potentially be disrupted by the mutations in PDE3A. It will only cause trouble in cells that produce the molecule, of which there are not many types.

"But if you think about it," he says, "they're just the types of cells you'd expect to find associated with Bilginturan's syndrome."

He stops talking. He's finished the sentence, finished his idea, but it still feels like he's stopped in the middle of something. We've been talking for an hour or so and suddenly neither of us knows what to say.

Fred looks at me. "Well," he says, "I guess that's it."

I can't think of any more questions. In an hour, or in the morning, I'll remember something I should have asked. But at the moment I'm all asked out.

The project is coming to an end, which is nearly unthinkable.

Philipp has just gotten married, and in about a month he is leaving for Harvard to take up a postdoctoral position in the lab of John Rinn. There he will continue to work on the functions of long noncoding RNAs, such as the molecule produced at the *CISTR-ACT* sequence.

Sylvia and Atakan will stay on campus, or at least in Berlin. But the laboratory they have called home for so many years will probably no longer exist, and neither is completely sure what lies ahead.

"Until this paper was finished, this project, I haven't been able to think any farther," Sylvia says. "I'd like to get back to genetics, or maybe do something in neurobiology." She'll probably keep teaching at the Charité, where she currently leads a course attended by eight medical students. She is guiding them through case histories, trying to help them find a direction for their own studies and careers. But it's time for a change.

Atakan was the member of the lab who had started out in a German "Realschule", trained as a technician and kept advancing to the next step, and the next, until he'd attained his PhD. He seems equal to any challenge. "What's next?" I ask him, "A professorship?" He laughs – it's not in his current plans. Maybe they'll give him a state-of-the-art sequencing facility to run.

So many others have drifted in and out of this story: Okan and Hakan and Jens and Thomas, Yvette and Astrid and Irene and Eireen. I'd like to know how things will turn out for each of them. But as the lab is disbanded, this time will fade into the background and remain only as a short, likely very interesting phase of their lives. Something brought them here; their stories became briefly intertwined, and now each of them has to let go .

And the long relationship with the Turkish family will surely dissolve as well. Fred plans to take some of us back to Karamat in the fall, to bring that part of the story to some sort of satisfying conclusion. Without this latest paper there might be no reason to return. The long relationship between the scientists and the family probably would have simply faded away without any real conclusion, to become a curious part of the family lore. This is a much better way to round things off. It's doubtful that there will be a true cure for this and many other genetic diseases for a long time. But the first step, which is to identify the problem, has already been taken. And at least there is a treatment that can keep the family alive.

This book and this story began with a question posed by Cafer's father, like a message for help, sealed in a bottle and thrown into the sea. Fifty years later, Fred will finally be bringing back the answer. It's the kind of thing that needs to be done in person.

Philipp Maass

Another visit will cause all these paths to cross once again. All of our lives have been enriched by the encounter. But we'll never fully learn how it will affect the family's future. They will go their way and each of the rest of us will go ours. That's just how things are at the end of a tale: you have to let the characters go.

But on this Friday the 13th of February, there's still something to resolve. The snow is gone, leaving the ground moist, and from it ascends the scent of the coming spring. Fred has sent off his seven-word mail to *Nature Genetics*, then waited around his office for a while, then has finally decided to go home. He lives here in the village of Berlin-Buch, a few minutes away by bike, and any day now you'll see him pedaling along again in a white lab coat, with his bike clips on.

At home he walks through the door and drops his keys on the table. He can hear his wife Ursula puttering around somewhere. In a minute he'll go find her. He puts down his bag and there across the room, his computer displays a screen full of stars.

Should he check his e-mail one more time? No, he's done enough for today. The Andromeda galaxy beckons. Oh, what the hell, he thinks and walks over and nudges the computer mouse. Up pops his e-mail, and what does he see but a response from the editor of the journal.

Dear Fred,

Thanks again for checking in. Although Reviewer #1 is still requesting additional experiments, I am happy to say that the editors have agreed to accept the paper in principle and that we will not require any further experiments…

There's more, but he will read that later. This is not the official letter, which will come in a few days – but it's the best possible answer. For a moment, right here and now, he feels the weight of all those years slip from his shoulders. He can set down the burden he's carried for so long; he can stop pushing and pleading and fighting for money. The work is not over, in science it's never completely over, but sometimes there comes a moment to savor that makes it all worthwhile.

In a minute or two he'll forward the mail on to the group, to everybody he's dragged along on this crazy quest, the whole lot of Sancho Panzas who have been enlisted into his personal, non-hypothesis-driven, Quixotic crusade.

But he won't tell the others just yet. For this one moment, the reply belongs to him, and him alone.

So just for a little while, he's simply going to sit here and enjoy it.

Note: photographs for Chapter 21 by Philipp Maass, Maj Britt Hansen, David Ausserhofer (p.22), Christiane Gross (p.22)