



Felix in Hospital. For the gene therapy a big intravenous catheter is needed. To allow Felix to move around in the way he is used to the infusion line had to be extended substantially

# Hey, **W<sub>H</sub>A<sub>T</sub>'S** up?

*It is night, the door to the bedroom is open. Suddenly Felix's father awakes, was there a noise? A cough, or even a choke? Quickly the parents check on their son. He is sleeping calmly in his crib. He seems to be breathing freely and there is no sign of blood on the pillow. Felix had Wiskott-Aldrich Syndrome, until he was successfully treated with a gene therapy in 2009.*

*His parents remember all the sleepless nights.*

MAXIMILIAN WITZEL



- 1 By using the technique of stem cell apheresis, the stem cells are harvested out of the peripheral blood, while the rest of the peripheral blood is given back. This procedure is highly time consuming and very exhausting for children, parents and doctors alike
- 2 Felix was always an agile child. He learned to walk in the same way his friends did, but in contrast to them, any downfall could have a devastating outcome. For example, after Felix fell on his mouth his whole face started to swell because of the hematoma
- 3 There is only success of the treatment, if parents, doctors and nurses act in concert. The family gives the children a small piece of normality in the hospital. (Felix playing hide- and-seek with his dad).
- 4 Felix has his own mind on how the new gene should find his way into his cells
- 5 The picture was taken during a visit in Munich. After the successful gene therapy Aaron is able to live a normal life. A couple of months ago he started to visit preschool

Their greatest fear was that Felix could suffocate from a nose bleed. The memory of the nights when they tried to stop four-hour nosebleeds with wet tissues, cool packs or nasal sprays is deep and hard to erase. The blood would flow in sync with his heartbeat out of his nose, sometimes only the ENT-doctor could stop the bleeding with a nasal pack or a transfusion of platelets. As a toddler Felix had infections conspicuously often. He frequently suffered from middle-ear infections or severe shingles on his face.

Change of scene. At lunch time the phone rings. Lawrence, the son, has fallen from his chair and has been unconscious since then. He opened his eyes briefly but did not regain consciousness.

Lawrence's mother rushes to the kindergarten. Last night Lawrence had a nosebleed, they don't know how long his nose was bleeding or how much blood he lost. In the morning the mother knew something was wrong, because he was unusually quiet and pale. Now they are very concerned about their son's health. Was the unconsciousness caused by the loss of blood? In the past it took them over 6 hours to stop his nose from bleeding; or could it be internal bleeding in his stomach or brain?

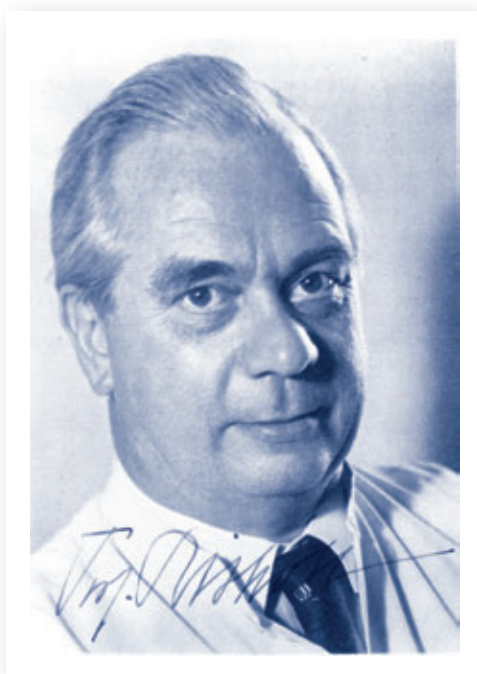
Lawrence and his brother Matthias have XLT. XLT stands for X-linked thrombocytopenia, a type of Wiskott-Aldrich syndrome where the patient only experiences the symptoms of thrombocytopenia. XLT is often described as a

mild version of Wiskott-Aldrich syndrome. The word mild doesn't fit in with the level of suffering that the families experience.

**Even gently bumping into things or just normal body movements could cause heavy internal bleeding, which contrasts with the natural urges of children to run and play.**

Change of Scene: Aaron was diagnosed with Wiskott-Aldrich Syndrome when he was only 6 months old. After learning about WAS we knew our son would not live a normal life and might even die. Aaron spent most of his life in and out of the hospital. Infections, vasculitis, nosebleeds that lasted many hours, numerous blood and platelet transfusions, IVIG treatments....noth-

ing seemed to work long enough to keep him well. When Aaron turned 2 years old we needed to make a medical decision and had to decide if a bone marrow transplant was an option for him. With no available bone marrow donor match for Aaron we needed to find a way to save our son's life. We learned about Professor Klein and heard of a clinical trial that was taking place in Hanover, Germany. We contacted Professor Klein and he agreed to meet us at Children's Hospital Los Angeles where he was speaking in September 2009. That was the beginning of a new life for our son. Aaron had gene therapy in December of 2009 in Hanover, Germany. Today Aaron is healthy and is living the life of a normal 4 year old boy. Aaron just started preschool, something that Wiskott-Aldrich Syndrome would have prevented him from doing. No more infections or transfusions, and no more hospital visits. Thank you, Professor Klein and gene therapy for saving our son's life.



1939-1945 and 1948-1967 Prof. Alfred Wiskott was dean and director of the Dr. v. Hauner Children's Hospital. In 1937 he wrote for the first time about a family with only boys affected. He suggested Morbus Werlhof, a chronic idiopathic thrombocytopenia, to be responsible for the pathology

## **WAS- HISTORY**

The German Pediatrician Alfred Wiskott (1898-1978) and the American Robert Anderson Aldrich (1917-1998) discovered the syndrome which is named after them.

Dr. Alfred Wiskott was dean of the faculty and the head of Dr. von Hauner Children's Hospital from 1939-1945 and 1948-1967. In 1937 he first described the triad of symptoms: bleeding from thrombocytopenia, susceptibility to infection and eczema. Three boys in the one family were affected by those symptoms and they all died in their first few months of life. The six sisters were healthy.

Wiskott assumed that the symptoms were caused by their genes. In a private discussion he told a hospital colleague that he was afraid because of the Nazis to call it a genetic disorder as he didn't want to put the family in danger. After the war on 1st January 1948 he was appointed as the dean of faculty.

The American scientist and pediatrician Robert Anderson Aldrich examined the family of an initial patient with similar symptoms.

All affected boys showed ear infections, eczema and bloody diarrhea. Retrospectively looking back at 4 generations 16 out of 40 boys of the affected

family died as infants. Female family members appeared healthy. Aldrich concluded an X-linked autosomal recessive trait of inheritance. In 1994 Derry et al. identified the WAS gene opening a new area of molecular diagnostics. Mutations could be seen in WAS Patients and XLT Patients. Today more than 440 mutations of the WAS gene are known.

In 2009 Prof. Klein and his team developed the very first gene therapy for Wiskott-Aldrich Syndrome which will be discussed in this article.

## **WAS - CAUSE OF IMMUNODEFICIENCY AND BLEEDING DISORDER**

The incidence of classical WAS is estimated to be 1-4/100 000. It is inherited via the X-chromosome. The location of the gene defect can be found on the short arm of chromosome X (Xp11.22). The gene continues across 12 exons and encodes the WAS protein (WASP). WASP is an important player in the immune system. A lack of WASP leads to a disturbance in the production of antibodies. Furthermore the cellular innate and adaptive immune function is affected and does not function correctly. WASP is a key factor in the activation of the immune system and plays an impor-

tant role in transmitting information between the divergent leukocytes types involved during immune response. In addition WASP influences the motility of immune cells.

Thrombocytes (platelets) are essential and the starting point for the coagulation cascade. Upon injury of blood vessels they get attached to the blood vessel's wall (adhesion) or get attached to each other (aggregation) and thereby stop the bleeding. Furthermore they build a net for other blood cells. All these aspects combine to stabilize the injured vessel. Then thrombocytes produce certain factors that amplify the coagulation cascade and therefore increase the stability of the injury.

One of the classical features experienced by WAS/XLT patients is thrombocytopenia and this is combined with extremely small thrombocytes. The pathogenesis of the thrombocytopenia, which is not transmitted via antibodies, is still unclear.

In addition to decreased numbers and dimension of thrombocytes due to the defective WAS gene there is also a thrombocytopathy, meaning a defect in thrombocyte function. We know that the thrombocytes of WAS patients have decreased adhesion functions and produce less factors that fosters the coagulation cascade.

## WAS – CLINICAL MANIFESTATIONS

The classical Wiskott-Aldrich syndrome is an immune deficiency that is characterized by the three following clinical features: recurrent infections, thrombocytopenia and eczema.

The clinical manifestation of these symptoms is highly variable. Patients can suffer from isolated thrombocytopenia (XLT) or have severe opportunistic infections such as deep abscesses, erysipelas osteomyelitis or recurrent otitis. Additionally, WAS patients tend to develop tumors of the lymphatic system that often evolve after an infection with the Epstein Barr Virus. The initial clinical symptoms can occur shortly after birth, including the patient exhibiting petechial bleeding of the skin, large hematomas or eczema of the skin. When Felix was born his whole body was laced with multiple small hematomas: *“He looked like a small puma baby”*, that is how his family describes it. *“He was heavy at the time of giving birth.”* *“In some cases after an extended labor the head and the shoulders of the baby can develop hematomas”*, his mother was told. *“But hematomas covering his body completely was too much.”*

In other cases the first symptom can be bloody diarrhea, caused by an autoimmune infection of the bowel. In Felix's case there were also concerns that he might have been suffering an autoimmune disease of the bowels, but then it was found that the reason for the bloody diarrhea was a low platelet count.

Other clinical manifestations of WAS can be an anemia or the degradation of certain subgroups of leukocytes triggered by autoimmunity.

## WAS – DIAGNOSIS

To diagnose a rare disease is often a difficult and time-consuming task. Many barriers have to be overcome. First, diagnoses of diseases that are much more common have to be ruled out. For example in the case of postnatal thrombocytopenia the first suspicion would be the presence of maternal antibodies against the child's platelets. To prove this hypothesis or to rule it out usually takes a time period of 3-6 months. That's why the median age at the time of diagnosis of WAS is 20 months. The

only exception is in families with several children with WAS, because a family history often means an awareness that leads to a quicker diagnosis.

One positive side-effect of the evolution of social networks on the internet in the past 2-3 years is that families with children with rare diseases can share their experiences. These days it is not only the knowledge and experience and skill of the medical specialist that alerts to the possibility of WAS, but more and more often it is the research and input of informed parents that leads to the diagnosis.

After three years of misdiagnosis and sorrow the diagnosis of WAS was proven in Felix's case.

*“Finally we had the certainty, but now we felt a new tremendous fear: Children with WAS often do not live longer than 10 years”*, Felix's parents report.

## WAS – STANDARD THERAPY

Prevention and treatment of infections and hemorrhages are the main aspects of conventional therapy for WAS. In cases of recognized deficiency, intravenous administration of immunoglobulin or platelets is required. Some patients with severe thrombocytopenia and recurrent bleeding undergo splenectomy (removal of the spleen). Today Felix and his parents are glad they did not take the risk of splenectomy. After splenectomy an augmented, lifelong risk exists for severe infections especially from pneumococci bacteria. Life-long treatment with oral antibiotics is recommended.

Until the advent of gene therapy for WAS a bone marrow transplant was the only curative treatment. Bone marrow transplants allow the replacement of a patient's hematopoiesis by one of a healthy, matching donor, known as an allogenic bone marrow transplant.

For allogenic bone marrow transplants to be performed a nearly complete eradication of the patient's own stem cells by means of chemotherapy or myeloablative chemotherapy is necessary. Myeloablative chemotherapy is associated with a marked morbidity and mortality. In addition graft-versus-host disease (GVHD) is a common complication after stem cell transplant.

Immune cells of the donor (graft) recognize the recipient's cells (host) as foreign. Transplanted immune cells can attack and cause harm especially in

skin, liver or gastro-intestinal tissue. The degree of matching at HLA loci of donor and recipient correlates with the intensity of GVDH, morbidity and mortality.

## WAS – GENE THERAPY

Prof. Klein and his team developed the first gene therapy for patients with Wiskott-Aldrich Syndrome. Described in simple terms the patient's own stem cells are collected, genetically corrected and re-transfused.

Patient's stem cells are modified with the help of a viral vector. By this means an optimized version of the WAS gene is introduced into patient's cells. After re-transfusion the corrected cells need to engraft in patient's bone marrow. To facilitate this engraftment chemotherapy is administered. However chemotherapy is reduced to 50% or less in comparison to the myeloablative chemotherapy used for bone marrow transplant. So, in gene therapy the patient's hematopoiesis is not completely knocked out. Severe infections due to chemotherapy did not occur in Prof. Klein's gene therapy trial.

Human bone marrow stem cells can be collected either directly from bone marrow or from peripheral blood.

To collect stem cells from peripheral blood treatment with granulocyte colony-stimulating factor (G-CSF) is necessary. G-CSF is a growth hormone. After treatment for several days stem cells are mobilized from bone marrow and appear in peripheral blood. There it is possible to collect stem cells via aphaeresis. Aphaeresis is an ordeal for patients and their parents. Felix's mother remembers:

*“You have to lay still for 8-10 hours without moving, blood is drained from one line, stem cells are collected and the remainder is returned via the other line. This was very, very exhausting.”*

Gene therapy for WAS offers several important advantages when compared to standard therapy. For example it is not necessary to find a matching donor, a process which is expensive and often without success. Infections due to myeloablative chemotherapy, high morbidity and mortality, do not occur to the same degree with less intense chemotherapy. Furthermore GVDH does not occur, a complication with high morbidity and mortality.

After introducing a corrected version of WAS in patients' own stem cells the new generation of blood/ immune cells are able to express the WAS protein. WAS expression allows reconstitution of the patient's immune system. The patient is phenotypically cured.

A major risk of gene therapy is mutagenesis i.e. the development of hematological malignancies like leukemia or MDS caused by the insertion of the viral vector in sensitive genomic regions. From an ethical point of view gene therapy of today can only be offered to those patients with complete WAS Syndrome, poor prognosis and for whom bone marrow transplant is not feasible.

A matching donor for Felix was not available. *"If you know, that your child will die without therapy in 10 or 12 years, you do not fear gene therapy as inserting a foreign gene in your child's body. The only thing you focus upon is that a healthy gene is introduced and that it works!"*, Felix's parents explain.

*"Today Felix can go to school, he is healthy, he participates in daily life, he laughs, blusters and plays."* Felix's parents are proud. *"When Felix tells me what kind of job he would like to do when he is grown up, I am overwhelmed with emotions, because I know, that thanks to gene therapy, he has a true chance to grow up."*

The first gene therapy trial for WAS assesses feasibility, safety and efficiency of this new treatment option. Patients

are under close follow up in order to react quickly to possible side effects.

Until the 3rd year after the therapy patients come every 3 months for follow up visits to Munich. A follow up visit always includes a thorough evaluation of the medical history since the last visit and physical examination, a major blood collection and bone marrow collection. Many of the families come from far away undertaking great effort and frequent, exhausting journeys.

## WAS – OUTLOOK

The Wiskott Aldrich Gene Therapy trial initiated by Prof. Klein and colleagues at the Hannover Medical School was the first gene therapy trial for WAS worldwide. Currently 10 patients have been treated in the WAS gene therapy trial.

*"New therapies always harbor risks"*, Prof. Klein states.

In gene therapy trials for X SCID (severe combined immune deficiency) in London and Paris, 5 out of 25 patients developed leukemia. Furthermore, in a gene therapy trial for chronic granulomatous disease (CGD), 2 of 3 patients developed a myelodysplastic syndrome (MDS) which can eventually lead to leukemia itself.

In the WAS gene therapy trial 3 out of 10 patients have developed leukemia, but all of them are doing better than before gene therapy. To improve safety

of gene therapy for WAS, a new, safer but at the same time efficient vector system is being developed. The start of a new gene therapy trial for WAS is planned for 2013.

*"Whenever we stop improving, we will inevitably fail"*, says Prof. Klein, winner of the Gottfried-Wilhelm Leibniz Prize from the German Research Foundation (2010) and the William Dameshek Prize from the American Society of Hematology (2011) for his work on WAS gene therapy.

New gene therapy vectors with improved safety profiles might allow gene therapy to be offered to patients with less severe phenotypes i.e. XLT like Lorenz and Matthias.

## WAS – NETWORKING

Rare diseases require an efficient communication between affected families, physicians and nursing staff.

A tightly-knit network with a means of taking short cuts in communication between different institutions, families and doctors might accelerate the initial diagnosis and therefore therapy and overall prognosis.

Today we know that the chance to reverse the WAS phenotype by gene therapy is dependent on the age of the child at the time they receive gene therapy - the younger the better.

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