Detective work reveals new secrets about rheumatoid arthritis

For a long time, some aspects of the genetics of rheumatoid arthritis perplexed scientists. Different genes were of different importance to different people. When Robert J. Winchester and Peter K. Gregersen managed to understand why, they found a first clue as to how the disease can develop. With this knowledge as a base, Lars Klareskog later developed a hypothesis describing how the most common form of rheumatoid arthritis develops, through the interplay between genes and environment. According to this hypothesis, the painful joint disease starts with modifications in a totally different part of the body: in the lungs.

Tens of millions of people all over the world suffer from rheumatoid arthritis. Over the course of the disease, the body’s immune system attacks the joints, which swell and become sore and rigid. Gradually, the inflammation destroys the cartilage and the bone; the joints deform and movement becomes difficult. Generally, a rheumatic person suffers from chronic pain.

Today, many patients with rheumatoid arthritis are almost free of symptoms, thanks to effective treatments. But not everyone responds to the therapy. As the understanding of rheumatoid arthritis has grown, scientists have realised that the disease may have different roots. In order to be able to develop treatments that help all patients, and maybe even prevent the disease, more detailed knowledge is needed.

The scientists awarded the 2013 Crafoord Prize, Robert Winchester, Peter Gregersen and Lars Klareskog, have, through systematic detective work, characterized the most common form of rheumatoid arthritis. To begin with, Winchester and Gregersen sorted out parts of the complicated genetics of the disease. Subsequently, Klareskog built on Winchester’s, Gregersen’s and others’ work, resulting in a detailed hypothesis as to why the immune system attacks the joints. It is a complex interaction between genetic inheritance and environmental influence.

Figure 1. Every cell carries so-called HLA proteins on its surface. Those proteins flag parts of the cell to immune cells. If these parts come from a healthy cell, the immune cell will just pass by. If the cell they come from is diseased, the immune cell will alarm the rest of the immune system, which will eliminate the cell.
Let us unravel the history behind the 2013 Crafoord Prize. It starts during the past century, when attempts to transplant organs between different individuals ramped up.

**A human recognition factor**

The first successful kidney transplant was conducted during the mid-1950s, between identical twins. But when surgeons tried to transplant organs between genetically different people, they often failed. The recipient’s immune system prevented the transplant; it reacted to another person’s organ as it reacts to pathogens like bacteria or viruses. Doctors became curious: why?

They discovered that every human cell carries a protein on its surface called human leukocyte antigen (HLA). Today we know that those proteins are like hands sticking out from the cell. The HLA proteins always hold parts of the cell in their hands. Immune cells – which constantly patrol the body – survey the content of the HLA hands. As they mature, immune cells learn to distinguish and tolerate everything that belongs to the body. They therefore can differentiate between health and disease. If a cell has been infected, for instance, by a virus, HLA proteins will hold parts of that virus in their hands. Immune cells will then immediately recognise that there is an unwelcome intruder and initiate an attack (figure 1).

When scientists investigated why transplantations almost never succeeded, they discovered that the HLA hands differ between people. There are plenty of different HLA variants. Everyone inherits one set of HLA variants from their mother and another set from their father. As the immune system develops, it learns to recognise its own body’s specific combination of HLA proteins. In a transplant procedure, another person’s organ almost always has different HLA proteins from the ones in the body receiving it. This is why the immune system reacts and rejects the organ.

So how is all this related to rheumatoid arthritis?

**Differences between various ethnic groups are a mystery**

The risk for developing rheumatoid arthritis can partly be inherited. Some families are more haunted than others. When scientists were looking for the genetic cause in the 1960s, they realised that differences in HLA mattered. They discovered that certain HLA variants increased the risk for the disease, but others were protective. However, different genes seemed to play a different role among different ethnic groups. An HLA type called DR4 is connected to an increased risk among Japanese people, Hispanic people and Caucasians. But for African-Americans and Israeli people with Jewish ancestry, for example, HLA DR4 does not have any effect at all. However, these populations have an increased risk if they carry a different HLA type: HLA DR1.

These differences were a mystery to scientists. Why were a variety of genes involved, and why did they not always have an effect? Some scientists thought that this was a red herring. They thought the genetic cause was something else, and that the HLA genes just happened to be inherited together with these as-yet unknown genes.

During this time of confusion, Winchester started to study the connection between HLA proteins and rheumatoid arthritis. At the end of the 1970s, he helped carry out a scientific study that implied a structural similarity between HLA variants that increase the risk for rheumatoid arthritis. When he established his own team, including Gregersen as a young researcher, Winchester continued to explore the world of HLA proteins using a variety of tools, including special antibodies.
In their natural environment in the body, antibodies are a part of the immune system. They stick to intruding pathogens, and this is a signal to the rest of the immune system that those pathogens should be eliminated. But scientists also use specifically designed antibodies as molecular fishing rods in their research. They develop antibodies that stick to certain structures in the body. If the antibody gets a “bite,” the scientists know that this special structure is present in their sample. In order to be able to trace the antibody, they make the antibody fluorescent or radioactive.

Among other things, Winchester and Gregersen utilized antibodies that recognised a specific part of HLA DR4. With this antibody, they went fishing in samples from patients suffering from rheumatoid arthritis. Surprisingly, they discovered that the antibody also got a bite in samples from patients that they were certain did not have any HLA DR4.

Many of those patients were instead carriers of the HLA DR1 type that increases the risk for rheumatoid arthritis. Since the antibody recognised both HLA types, the conclusion was that HLA DR1 must contain a structure similar to one in HLA DR4.

This was an important clue. But it would take the scientists a few years to discover exactly what the antibody recognised and how all this was connected on the molecular level. At the time, they thought they had found what they called “Cinderella’s shoe”. But in order to understand how Cinderella’s shoe fits into this story, we need to dive deeper into the world of proteins and explain how proteins like HLA are constructed.

A building kit of amino acids

Proteins are made out of amino acids that are linked together in long chains. One single protein can contain hundreds of amino acids. The amino acids are like a kit of children’s building blocks with 20 different types of bricks. Depending on how the 20 different types of amino acids are combined, different proteins with different functions are formed. The long chains fold into something that resembles a tangled ball of yarn, to form a protein.

To understand why certain types of HLA DR4 and HLA DR1 looked similar, Winchester and Gregersen needed to know the order of the amino acids in the two long chains that compose the proteins. Luckily, there is a shortcut to such information — a shortcut that goes through the body’s genes. Each gene contains the blueprint, or code, for how amino acids should be combined into a specific protein. If scientists can read that code, they know exactly which amino acids build the protein and in what order they are linked together.

Today, machines can map all of the genes from one human being within a few days. During the 1980s, it could take several years to read the code for one single gene. But for Winchester, Gregersen and their co-workers, it was worth all the trouble. When they finally could compare various HLA types, they found that all proteins connected to an increased risk of rheumatoid arthritis had significant similarities around position number 70 in one of the amino acid chains. Their conclusion: this part of the protein must be of particular importance – this must be the common denominator.

Scientists find Cinderella’s shoe – but what does the foot look like?

Just one year later, Winchester and Gregersen realised why amino acids neighbouring position 70 in the HLA protein have an impact on rheumatoid arthritis. At that time, other researchers had managed to develop a detailed three-dimensional image showing how the amino acid chains of HLA
proteins are folded. In figure 2, you can see an illustration of this. The chains form like a flat floor, which is lined by two spiral-shaped structures. Together they form an oval pocket. This pocket is the “hand” of the HLA proteins. Different parts of cells, bacteria or viruses get stuck in this pocket, and the patrolling immune system continuously checks the contents inside.

Winchester and Gregersen realised in 1988 that the amino acids in positions 67–74 are located in the spirals forming the pocket. Those amino acids are therefore crucial to the pocket’s shape. And this is where Cinderella’s shoe enters the story. Earlier we drew parallels between HLA proteins and hands sticking out from the cell. However, since the form of the pocket seemed important, Winchester and Gregersen instead compared it with Cinderella’s shoe. The scientists quote the story and write:

“Hurrying after Cinderella, the prince could find only her glass slipper”.

Cinderella’s shoe fits only her foot, and the fairytale prince uses the shoe to search for her. In the same manner, the scientists thought that the particular shape of the HLA pocket could be used in the search for the molecular equivalent of Cinderella’s foot. A molecule fitting into this pocket must somehow be involved in the development of rheumatoid arthritis. Winchester and Gregersen called this unknown molecule “factor X”. In scientific language, the pocket and its shape are called “the shared epitope”. An epitope is a specific structure recognized by an antibody.

A Swedish database yields an important clue

It would take almost 20 years before researchers caught the scent of factor X. Here is where this year’s third Crafoord Laureate enters the story. Lars Klareskog and his team were curious to see if there was any connection between HLA risk genes and the already known association that smoking increases the risk for rheumatoid arthritis. In order to study this, Klareskog and his co-workers used a large Swedish registry established in 1996, in which rheumatologists have collected detailed information on people suffering from rheumatoid arthritis, concerning both their disease and their lifestyle.
Klareskog and co-workers used the registry’s information to see if smoking influences the effect of HLA risk genes. The answer was unequivocal: YES!

In a study in 2004, scientists divided rheumatics into two separate groups: so-called seropositive and seronegative. In the seropositive group, the scientists found the clear relationship between HLA risk genes and smoking (figure 3). People who have only one of the risk factors, who carry a risk gene or who smoke have a modest (about 2.5 times) increased risk for developing rheumatoid arthritis. However, the situation is totally different if a person both carries risk genes and smokes. Smokers with one risk gene have a 5.5 times higher risk for rheumatoid arthritis. Smokers who have been unlucky enough to inherit risk genes from both their mother and father will be plagued 15.7 times more often with the disease than non-smokers without risk genes.

This was a groundbreaking study. In addition to this, in a follow-up study, Klareskog and his co-workers found another interesting relation: the connection between HLA risk genes and smoking is even stronger if a person simultaneously has antibodies against so-called citrullinated proteins in their blood.

Can smoking shape Cinderella’s foot?

Citrullinated proteins exist naturally in the body and are formed when a specific amino acid, arginine, is modified chemically to form another amino acid, citrulline. Other scientists have discovered that certain rheumatics have antibodies against those proteins in their body. Klareskog and his team connected those antibodies to HLA risk genes and smoking. A smoker who has antibodies against citrullinated proteins and who simultaneously carries a double set of HLA risk genes has a more than 20 times higher risk of developing rheumatoid arthritis.

Klareskog’s research showed that smoking increases the amount of citrullinated proteins in the lung and that the more a person has smoked, the larger their risk to develop antibodies against those proteins. Taken altogether, this implies that the immune systems of the majority of rheumatoid arthritis patients recognize citrullinated proteins as something foreign. Those proteins could carry the secret of Cinderella’s foot: they may be the “factor X” that fits into the pocket shape of the shared HLA epitope.

A hypothesis that many scientists are exploring is that rheumatoid arthritis can develop in two steps. First, smoking results in citrullinated proteins in the lungs, and those proteins fit perfectly into the shared epitope of HLA. The HLA proteins expose citrullinated proteins to patrolling immune cells. Through this process, the immune system begins to recognize citrullinated proteins as something foreign that should be repelled. This phase can persist for quite a while. People can carry antibodies against citrullinated proteins for many years before they experience any problems with their joints. But suddenly something happens. For some reason – maybe due to some injury – citrullinated proteins also form in the joints. Since the immune system already recognizes those proteins as foreign, it will attack and the joint-destroying machinery is set in motion. Research shows that if Sweden were smoke-free, every fifth case of rheumatoid arthritis would be prevented.
Hence, rheumatoid arthritis can, in certain cases, begin in the lungs. But this is not yet scientifically proven. Other explanations are possible as to why smokers with HLA risk genes often have antibodies against citrullinated proteins in their blood. During the past few years, scientists have also found an additional gene that is involved in this variant of rheumatism, which might be a new key to the disease. The last word on Cinderella’s shoe thus has not yet been said. But the discoveries awarded the 2013 Crafoord Prize have fundamentally contributed to the understanding of rheumatoid arthritis.

This year’s Crafoord Prize strongly illustrates the power of research to map a disease in a systematic way and get results. Through large-scale studies, the Laureates have identified interesting correlations and subsequently explored those correlations in the smallest molecular detail. They have fitted one piece of the puzzle to another to create a model for how the most common form of rheumatoid arthritis might develop.

Approximately 70% of all people with rheumatoid arthritis suffer from this variant related to citrullinated proteins, which often progresses aggressively. The negative effects of inflammation have led doctors to start treating the disease at an earlier stage, especially if a person carries antibodies against citrullinated proteins. Treatment is often effective, but does not help all patients. Hopefully, the discoveries behind the 2013 Crafoord Prize can contribute to even better treatments, to prevent fewer patients from suffering from the pain of their disease.

**POLYARTHRITIS**

Polyarthritis is a collective term for rheumatic diseases that involve inflammation of several joints in the body.

The commonest form of polyarthritis is rheumatoid arthritis. The immune system identifies joints in the sufferer’s own body as foreign, and breaks them down. Women are more often diagnosed with rheumatoid arthritis than men.

Gout is a form of polyarthritis in which uric acid crystallises and is deposited in the joints. The immune system reacts to the crystals and inflammation results.

People with psoriasis, a chronic skin disease, can also get inflamed joints. This is known as psoriatic arthritis. The joints of the back are sometimes also affected.

Bechterew’s disease (also known as spondylarthritis) also causes inflammation of the back joints. This disease usually affects men and the first symptoms commonly appear before the age of 40.

Reactive arthritis sometimes arises after intestinal or urinary infections. The disease can be caused by fragments from bacteria that have lodged in the joints.

People who suffer from the rheumatic disease systemic lupus erythematosus (SLE) can also get polyarthritis.

Juvenile arthritis (or juvenile rheumatoid arthritis) is a collective name for several different types of polyarthritis that affect children and adolescents.

Still’s disease is a particularly aggressive form of juvenile arthritis involving fever, rashes and polyarthritis.

**LINKS AND FURTHER READING**

More information about this year’s prize is available on the Royal Swedish Academy of Sciences’ website, http://kva.se/crafoordprize and at www.crafoordprize.se

**Lectures (video):**

Gregersen, P. K. (2010) Genetics, www.youtube.com/watch?v=cEQdnJWE1Cg


**Websites:**

Rheumatoid arthritis
http://www.medicinenet.com/rheumatoid_arthritis/article.htm
http://www.medicalnewstoday.com/info/rheumatoid-arthritis/
THE LAUREATES

Peter K. Gregersen
http://www.feinsteininstitute.org/Feinstein/Laboratory+of+Genomics+and+Human+Genetics

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http://ki.se/ki/jsp/polopoly.jsp?id=5763&i=en

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http://www.rheumatologyatcolumbia.org/rwinchester.html