The genetics of Crohn's disease decoded

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Through global cooperation, all the major laboratories active in research on Crohn's disease succeeded in obtaining a very precise genetic map of this inflammatory disease of the digestive system. The genetic heritage of some 75,000 patients was thoroughly examined. With 163 genetic variations identified, Crohn's is currently the best genotyped disease. A great hope for sufferers. But the research published in Nature magazine (1), which a team from the University of Liège's Giga Research collaborated on, also revealed the great complexity of a pathology that is undoubtedly the result of war waged by our body in the past against terrible microbes such as tuberculosis or the plague.

Petrus opened the door; a ray of sunshine slipped into the house and lit up the only room in the modest dwelling. Six corpses lay on the dirt floor: his father, his mother, his sister and his three brothers. It was the same macabre scene in the neighbouring houses: lifeless bodies everywhere, covered in buboes. That week, the plague had wiped out the whole village. The epidemic had arrived in the town two weeks earlier with a distant cousin who had returned from the war against the English. The carnage had begun several days later. Only Petrus had survived. "Why me?" he wondered amid all this desolation. He beseeched the Lord to provide him with an answer.

Pierre opens the door of Dr. Deliège's surgery. He has come to find out the results from a colonoscopy that was carried out the previous week at Liège CHU. He's anxious. He's been suffering from abdominal pain and chronic diarrhoea for several months now. He finally decided to see a doctor when he realised he'd lost five kilos. "You're suffering from a chronic inflammation of the intestine", the doctor told him, "Crohn's disease. Don't worry, it won't kill you. But I have to be honest with you, you can't get rid of it either. There is treatment available to relieve the symptoms, but when the disease evolves too quickly, we sometimes have to surgically remove part of the intestine". Pierre is bewildered: "I don't understand, I don't drink alcohol, I eat healthily..." "We still don't really know what causes this disease", the doctor replies. "There is clearly a strong genetic component. By comparing the genetic heritage of people with and without the disease, researchers have
identified 163 precise places on the genome that could be involved in the disease. It's possible that the genes that cause this chronic inflammation of the digestive system today, allowed our ancestors to escape the plague epidemics in the Middle Ages. A French researcher supports this theory. And a recent study published in Nature is grist to the mill.

**Studying 75,000 patients**

This study is an extraordinary scientific adventure. Several years ago, a number of laboratories specialising in the genetics of Crohn's disease attempted to be the first to discover one or more genes associated with the disease. A team from the University of Liège's Giga Research (led by professors Michel Georges and Édouard Louis) was in the race. Thanks to collaborations between several of the country's major hospitals, the team in Liège managed to recruit some 3,000 people for their study (1700 sufferers and 1500 non-sufferers as the control group). A statistically sufficient amount to isolate an initial gene that is clearly involved in Crohn's disease. In fact, our researchers revealed several variants in the same genetic sequence, one of which is present in 20% of people with Crohn's and in only 12% of healthy patients (read the article).

"But we realised that in order to go further, we would have to work on a larger number of patients", Professor Édouard Louis explains. "Four teams were working on this research: NIH in the USA, the Wellcome Trust Centre in the UK, Kiel University in Germany and GIGA in Liège. Despite the scientific competition, this happened very spontaneously, since it was in everyone's interest to work together. Three telephone calls and it was sorted." Three telephone calls to create a global science consortium (today, some 30 countries are involved) responsible for recruiting patients from the four corners of the world, performing a genetic analysis on intestinal tissue samples and recently published a very complete set of results in Nature magazine, relating to 75,000 patients this time. Just as spectacular in this study is the number of genetic variants revealed that are associated with Crohn's disease: 163, 71 of which were absolutely unknown! "We have doubled the knowledge we have on the disease. No other pathology is currently better genotyped than Crohn's", says a delighted Édouard Louis.

This important breakthrough was made possible by the vast meta-analysis (cross-checking and comparison) of 15 previous studies, including those carried out in Liège in 2008. But also thanks to the use of specific genetic equipment: a genetic chip.
(Immunochip) specially developed for the study of immune diseases: Crohn's, diabetes, psoriasis, rheumatoid arthritis, etc. The miniaturisation achieved in this type of equipment exceeds all expectations. A chip measures 12 cm² and can analyse the DNA of 12 patients, i.e. 1 cm² per patient. Millions of tiny beads, each containing single DNA strands, are attached to every square centimetre of the chip. The study tested 196,000 different ones, each of which contained a genetic variant (Single Nucleotide Polymorphism or SNP) likely to be involved in Crohn's disease. Once placed on the chip, the patient's DNA sample will recombine with the strands attached to the chip if the genetic sequence is the same, in other words, if the patient carries one of the 196,000 variants tested on the chip.

"We're not searching blindly through the three billion letters of the genome", explains Emilie Theâtre, a Giga Research researcher. "The sequences tested by the chip are either genes that have already been identified for their role in Crohn's disease, or genetic sequences that produce proteins known for their role in the inflammatory process of the digestive system, or genes associated with other inflammatory diseases such as diabetes or psoriasis." The study published in Nature confirms the genetic relationship of several inflammatory immune diseases: Crohn's, ulcerative colitis, diabetes, rheumatoid arthritis, psoriasis, etc. In some rare cases, patients can develop two or three of these diseases at the same time. And epidemiological studies show that some families are more affected than others by these inflammatory immune diseases: an uncle has diabetes, a nephew has Crohn's, a first cousin, develops psoriasis, a great-aunt had ulcerative colitis, etc. For instance, there is extensive genetic overlap between diabetes and Crohn's disease. Out of the 39 genetic variants identified in diabetes, 20 of them can also be found in Crohn's disease. In that case, how can it be that the majority of people only develop one of these inflammatory diseases? In reality, genetics only rarely explains a single disease. The medical destiny of identical twins (who have exactly the same genetic heritage) provides a good example. In the case of Crohn's disease, for instance, it is common for only one of the twins to develop the disease. The person's environment and history must also be taken into account.
As we said, the genetic map of Crohn's disease is now very complete: 163 variants are associated with the disease. The map is certainly precise, but none of these variants alone explains the disease, which clearly results from a "losing combination". But is it a combination of two, three, four, five genetic variants? Or more? Mathematically, there are an almost infinite number of possible combinations. The solution is beyond the reach of the current models. So much so, that this wealth of genetic information can't be used to develop diagnostic tools. In any case, the usefulness of developing a diagnostic tool is currently limited because there is no preventive treatment for the disease.

Instead, the aim is rather to understand the physiopathology of the disease in order to develop new treatments. The researchers are endeavouring to understand the molecular cascade that leads these genetic variations to the clinical manifestation of the disease. Thanks to the mouse knockout technique, for instance, it is possible to observe the consequence of precise genetic suppression. Another avenue consists of taking immune cells from the intestine, studying the genetic expression within these cells and seeing if these active genes correspond to one or other of the 163 known variations. But this type of research is very time-consuming. The first gene in Crohn's disease was identified in 2001 and we still don't really know how it causes the disease.
One of the immediate stakes of this work on the physiopathology of the disease is sorting between patients who develop a benign form of the disease (40%) and who should avoid the serious side effects of immune system suppressors, and those who develop the most severe form of the disease (60%) and who must be treated as quickly as possible.

**Crohn's, tuberculosis, leprosy and... the plague!**

As regards fundamental knowledge, the *Nature* study confirms an astonishing genetic link with certain relatively rare pathologies characterised by immunodeficiency. This is paradoxical, insofar as Crohn's disease is caused by an excess of immunity rather than a lack of it. Perhaps the paradox is not what it seems. There could be two phases in the development of the pathology. First of all, an immunodeficiency of genetic origin leads to the poor management of the microbial environment, especially of the intestinal flora, which translates into an abnormal microbial colonisation in the first lines of defence. The typical inflammatory immune reaction of Crohn's disease only occurs later, because the digestive system feels attacked. But the reaction is disproportionate; it causes more harm than good and destroys the digestive system.

The study also confirms a genetic link between Crohn's disease and infectious diseases like tuberculosis, leprosy, and even... the plague! This link was already suggested in 2003 by a French researcher, Jean-Pierre Hugot, in a study published in the Lancet. The first gene associated with Crohn's disease - Card 15 - is known for its role in immunity. And in the intestines of people with Crohn's disease, we often find traces of bacteria from the Yersinia family (Yersinia Enterocolita, cousin of Yersinia Pestis, the bacteria responsible for the plague). Jean-Pierre Hugot's hypothesis is as follows: in the Middle Ages, a certain number of people carried a particular form of the Card 15 gene which made them immune to the bacteria responsible for the plague. Petrus would carry this genetic mutation unlike all the other inhabitants in his village... Those, like Petrus, who survived the epidemics, had children and transmitted their genetic heritage to their descendents. Seven hundred years
later, Pierre inherited this gene but as he now lives in Liège, he is no longer in contact with the plague bacterium (which is still rife but not on an epidemic scale in the world's poorest regions, especially Africa). On the other hand, Pierre is often in contact with other bacteria from the Yersinia family (which colonise certain foods and are capable of resisting the cold temperatures of a fridge) but which are less dangerous than Pestis. And his immune system, which remembers the terrible epidemics of the Middle Ages, overestimates the dangers and reacts excessively. The inflammatory reaction in the intestine is disproportionate and turns against the sufferer. This is how natural selection may have enabled certain people to escape epidemics that destroyed half of the population of Europe in the 14th century, but has now caused the pendulum to swing back in the form of one or more autoimmune diseases (Crohn's, diabetes, etc.). And if we continue to follow the logic of natural selection, these new diseases are not likely to disappear, because they don't prevent sufferers from having children and perpetuating their genetic heritage. Man has practically eradicated the plague. But he will perhaps have to live for a very long time with Crohn's disease.

(1) Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Published in Nature online 01 November 2012.