CADASIL France

AGM of April 2 2016

The Medical Team's Presentations

On the occasion of the AGM of CADASIL France on the 2nd of April 2016, numerous members of the medical team participated in the afternoon, which was devoted to the medical presentations: Professor Hugues CHABRIAT (Head of the Neurology Department of Lariboisière Hospital, Coordinator of CERVCO, Centre of Reference for Rare Vascular Illnesses of the Brain and Eye); Professor Marie-Germaine BOUSSER; Dr Anne JOUTEL (Research Director at INSERM, in charge of biological and genetic research on CADASIL); Mrs Annie KURTZ (retired psychologist from Lariboisière's Neurology Department and member of the committee of CADASIL France); Dr Dominique HERVE (hospital practitioner, neurologist, in charge of CERVCO); Dr Eric JOUVENT (Assistant University Professor and practitioner at Lariboisière); Mrs Christina ROGAN, Sonia REYES and Aude JABOULEY (psychologists at CERVCO who work on the cognitive evaluation of patients, support them and their families, answer the questions of younger family members, etc.); Mrs Jocelyne RUFFIE (assistant of PRs BOUSSER and CHABRIAT); M Abbas TALEB (Clinical Research Attaché, in charge of the database for the follow up of patients); Mrs Stéphanie MOREL (Social Assistant of CERVCO); Mrs Nathalie GASTELIER (Project Leader within the Lariboisière University Hospital Department); Dr Carol PRIETO-MORIN, from the genetic laboratory directed by Professor TOURNIER-LASSERVE at Lariboisière.

1. Introduction by the President of CADASIL France

At the 2016 AGM of CADASIL France, the afternoon has been dedicated to the medical presentations, and it started off by Jean-Luc Airiau who paid tribute to all those who are working towards a better understanding and treatment of the CADASIL illness. He affirmed his confidence in the progress of the research and the willingness of the Association to contribute, however modestly, towards financing it. Although the time taken for the research seems long and the complexity of the discoveries makes the early hope of a treatment seem further away, the progress we are presented with each year does bring us hope, thanks to the dedication and contribution of the members of the medical and research teams.

2. Genetic Research (Dr Joutel)

During the last year, Dr Joutel has continued her work with her team, on studies preceding the clinical research. The aim is to understand the mechanisms of the illness in order to elaborate therapeutic strategies in conjunction with Professor Chabriat's team.

Three subjects are going to be broached: (a) the mouse models which are used to study the illness, (b) the very precise and complex mechanisms of the illness, which we now understand much better, and (c) the results of a pre-clinical trial.

(a) The importance of animal models in CADASIL

It is very important to be able to use animal models which reproduce the signs of CADASIL. These models allow us to better understand the illness, to discover the mechanisms of the

symptoms and to evaluate therapeutic strategies. Thanks to animal models, we can test different action strategies but no medical treatments.

Dr Joutel's laboratory has generated a first type of mouse model with certain characteristics called transgenic mice. These mice overexpress a NOTCH3 protein with the mutation of a patient.

The abnormalities found in these mice are the following: early in their development we find vascular deposits of NOTCH3 and of GOM. They also develop a reduction in the diameter of their cerebral arteries (which signifies that they are less capable of stretching), a cerebro-vascular dysfunction, and lesions of the cerebral white matter (which could correspond to the hyper signals detected in the MRI scans of patients).

An American team has also recently shown that these mice display a marker (a lowering of the threshold of the intrusive cortical depression) for migraine with aura. Almost half of the people with CADASIL display this symptom.

The advantage of this type of mouse model is that the symptoms appear sooner than in patients. However, we can question the relevance of this type of mouse model because it has several copies of the mutated gene *Notch3* and it over expresses the mutated protein.

In order to confirm what we have learnt from these transgenic mice, another type of mouse model has recently been produced thanks to a collaboration with a Belgian team. In this model, the mice have a mutated *Notch3* gene identical to that found in man, without over expression.

The ongoing analysis of these mice has confirmed the presence of vascular deposits of NOTCH3 and of GOM, a reduction in the diameter of cerebral arteries and the presence of cerebro-vascular dysfunction. This proves that these alterations are definitely linked to the mutated *Notch3* gene, even without over expression. The difference is that the functional abnormalities of the cerebral vessels appear at the age of 12 months, and so much later than in the transgenic mice. The analysis of the cerebral white matter of these mice is still in progress.

This confirmation is important in order to have at our disposal pre-clinical models for testing therapeutic strategies. We can be certain of having an evaluative tool when using CADASIL mice.

The symptoms and lesions that we see in mice are clearly linked to the mutation, even without forcing an over expression of mutated protein!

Using the mice we have studied for many years, we have decoded, symptom by symptom, the mechanisms and their causes.

Basically, it is the mutated protein NOTCH3 which is the cause, but it is the disturbances that it provokes which produce the symptoms:

- The functional abnormalities of the vessels (cerebro-vascular dysfunctioning) are linked to the accumulation of the TIMP3 protein which is itself caused by the accumulation of NOTCH3.
- The lesions of the white matter are in part linked to the accumulation of Vitronectin, provoked by the accumulation of NOTCH3.
- The shrinking diameter of the cerebral arteries is linked to a modification of the activity of the NOTCH3 receptor.

The "conductor" of these dysfunctions is the mutated *Notch3* gene, the carrier of CADASIL, which produces the mutated NOTCH3 protein. But its agents are different. There are other proteins which are at the origin of the symptoms.

The mechanisms which lead these other proteins, the normal ones, to cause abnormalities are complex. We must therefore act on this “conductor” at a therapeutic level rather than on its agents, as it would be complicated to act on each one of them and on the mechanisms that they provoke.

The aim of the therapeutic trials must be to stop the NOTCH3 mutated protein from diverting TIMP3, Victronectin and other proteins from their habitual role.

Now, thanks to new tools and collaborations, we can explain the abnormalities of the functioning of the vessels in the brain in molecular detail!

(b) Mechanisms of cerebro-vascular dysfunctioning

In a normal mouse, a sensory stimulation (for example, stimulation of its whiskers) leads to an activation of the neurons, which in itself leads to an increase in cerebral blood flow. We call this “neuro-vascular coupling”. In transgenic CADASIL mice, the neuro-vascular coupling is altered, i.e. the increase of cerebral blood flow in response to an activation of the neurons is lessened. Complementary experiments have shown that the CADASIL mice are incapable of increasing their cerebral blood flow in response to different stimuli.

Furthermore, experiments carried out on previously dissected sections of cerebral arteries have shown a diminution of myogenic tone in the CADASIL mouse. Myogenic tone is a very important property of small arteries; it defines a state of contraction of the artery in response to arterial pressure.

The term “cerebro-vascular dysfunction” is used to designate these abnormalities of cerebral blood flow and the myogenic tone of the cerebral arteries of the CADASIL mouse.

Dr Joutel’s team has shown that cerebro-vascular dysfunction in a CADASIL mouse is caused by an excess of the protein TIMP3.

Thanks to a very good collaboration with Dr Mark Nelson’s American team in the USA (within the framework of the transatlantic network, financed by the Leducq Foundation), and with Dr Stefan Rose-John’s laboratory in Germany, all the components working in the string of events which lead from the excess of TIMP3 protein to the cerebro-vascular dysfunction have been identified. Furthermore, it has been possible to establish a link between the abnormality of the responses in cerebral blood flow and the myogenic tone of the cerebral arteries.

Thus, it has been demonstrated that the abnormal accumulation of the TIMP3 protein inhibits the functioning of a protein called ADAM17 which controls the activity of a receptor which itself regulates the number of potassic channels at the surface of the smooth muscular cells of the vessel walls. These potassic channels, whose role is to regulate the voltage of the membrane of the smooth muscular cells, play a very important role in the myogenic tone. Thus, when there is an excess of TIMP3 the number of these potassic channels is abnormally increased, the myogenic tone is diminished, which limits the capacity of the cerebral arteries to dilate and so limits the increase in cerebral blood flow.

These are new indicators which are equally important in the understanding of both normal vessels and the regulation of cerebral blood flow.

These potassic channels are present in all other arteries, but only in a small quantity in the cerebral arteries. And there, by increasing these channels from around 30% to 50% it disturbs the functioning of these vessels in the initial stages of the illness.

There are some drugs which are able to block the activity of these potassic channels. However, using them in man is made extremely delicate because of the presence of potassic channels in and around the heart. Indeed, blocking these channels when they are situated around the heart could lead to very dangerous secondary effects.

We are therefore now able to explore and understand the symptoms at a very sophisticated level!

On the basis of results obtained with CADASIL mice, Professor Chabriat's team has undertaken a study of neurovascular coupling in patients with CADASIL. Encouraging preliminary results also suggest the existence of an abnormality in these patients' neurovascular coupling (see below).

(c) Pre-clinical Trial

A proof of concept has been established. That means that a type of molecule has been tested to show that it had an effect on the symptoms of CADASIL mice.

This is a pre-clinical trial because it is carried out with mice. The results are preliminary as they were reached at a stage when the mice had deposits of NOTCH3 but they had not yet developed cerebrovascular dysfunction.

This is a project which was run in collaboration with the Danish Lundbeck laboratory. A patent has been filed in order to protect this discovery and so that these partners have the rights to continue their work.

The molecule tested is an antibody which recognises that part of the NOTCH3 protein which is accumulating. The idea of testing an anti-NOTCH3 antibody came from work done in other illnesses where proteins accumulate in an abnormal way, such as in Alzheimer's, where an accumulation of the protein Amyloid and the protein Tau exists. In fact, about 20 years ago a researcher had the idea to immunise transgenic Alzheimer mice against the protein Amyloid. He obtained very spectacular results because both the number and size of the Amyloid deposits were considerably reduced in the treated mice. These results have since been confirmed by numerous other teams all over the world by using antibodies targeting the Amyloid protein.

This principle of so called "passive immunisation" is different from a vaccine (which consists of an injection of a small amount of a virus in order that the person himself makes the antibodies, which is therefore called "active immunisation").

This technique of injecting an antibody is now well-known. It is used in inflammatory illnesses. And we understand its toxicity. In the context of a trial, certain stages of toxicity evaluation could be shortened because this technique is well-known, and we know about the secondary effects. But this type of therapeutic treatment remains costly.

Several years ago, Dr Joutel created monoclonal antibodies targeting short sequences of the human NOTCH3 protein. Amongst these antibodies, one of them was identified as recognising the mouse NOTCH3 protein.

An initial study has been carried out to see if this antibody, when injected into a mouse, would bind itself to the deposits of NOTCH3 which were present, especially in the vessels of its brain.

We found that in the CADASIL mouse, several days after the injection, the antibody anti-NOTCH3 was able to bind itself to all the deposits present in the arteries and even in the capillaries (very small vessels), while a control antibody (recognising a different protein) did not have that effect.

For the pre-clinical trial, we injected CADASIL mice with antibodies from the age of 2 months up to 6 months with the same dose every week. As a result of this test we noted that the antibody anti-NOTCH3 resulted in the CADASIL mouse not showing any differences to a normal mouse (wild) in the amount of shrinking of the diameter of the brain arteries (shown by dissection of small pieces of the cerebral vessels), nor in the cerebral blood flow (increasing of the flow in response to stimulation), nor in the myogenic tone (contraction in response to pressure, especially in cerebral blood pressure). These parameters returned almost to normal following the injection of the antibody.

Another test was done giving one single injection to six month old mice. The results show that one single injection is not enough.

Contrary to all expectations, and despite the beneficial effect observed on the properties of the vessels, it has been proved that the treatment had no visible effect on the number of deposits of NOTCH3, which remained unchanged.

These are the preliminary results of a study taking place at a point where there are not yet any symptoms of the illness. We have discovered that the antibody anti-NOTCH3 can prevent them.

This preventative study has to be completed. We must also test to see if the antibody is effective when it is administered to CADASIL mice which are already showing signs of cerebro-vascular dysfunction.

This study must also be carried out on other types of CADASIL mice. We must also make sure that there are no side effects.

Finally, markers for what is happening must be found, so that we can better understand antibody anti-NOTCH3's mechanisms of working. These will be tested initially on the peripheral vessels outside the brain, which receive more of the antibody following an injection. We must also find ways of improving the rate of penetration of the antibodies into the brain, in collaboration with other research laboratories. In fact, when the antibody is injected into the mouse intraperitoneally (into the abdomen) a very low amount (about 0.1%) goes on to penetrate the brain. Techniques must be found to increase the rate of penetration into the brain.

In order to continue these studies, it will be necessary to look for financial aid from new partners.

Dr Joutel's team is made up of: Imane Haddad, study engineer, who is preparing a thesis on the proteomic aspect of brain vessels; Lamia Ghezali, a postdoctorate researcher who is devoting herself to the preclinical trial; and Julien Ratelade, who is working on the mechanisms of another hereditary illness of the small vessels of the brain. Valérie Domenga-Denier, assistant engineer, has for many years completed the laboratory team.

Carmen Capone, who was studying vascular dysfunction, has gone back to live in Italy. Céline Baron-Menguy, research engineer, who was working on the vessels, is going back to work in the provinces. Recruiting new people and finding ways to pay for them, are therefore going to be necessary.

Question from a conference member. What is the role played by the *Notch3* gene? Does it have an effect on the skin?

Dr Joutel explained that *Notch3* is principally found in the vessels, both of the brain and other organs. Its normal role was described by her team in the early 2000s: in the development of the

vessels, *Notch3* plays a part at the level of the smooth muscular cells of the vessel walls so that they acquire arterial characteristics.

During the course of adult life, this gene plays a part in the contraction of the vessel under pressure (the myogenic tone) and it controls the diameter of the vessel.

Other *Notch* genes belonging to the family of Notch receptors affect the skin.

Question from another conference member. Are the deposits in the vessels involved in the symptoms?

Dr Joutel explained that the big GOM deposits, made up of a lot of NOTCH3 and other proteins, are the most visible as they are the largest, but it is thought that it is the small intermediary deposits (oligomers) made up of 4 or 5 molecules of NOTCH3, which are toxic for the vessels and provoke certain symptoms. These small clumps are a stage in the formation of big deposits.

We should possibly not destroy these big deposits of GOM which are perhaps there to neutralize the intermediate agglutinations (small deposits).

Work is going to be done to test this hypothesis.

Question from Professor Bousser. With CADASIL, is a cerebral vessel dilated and at the same time limited in its capacity to dilate?

Dr Joutel indicated that there are actually two characteristics.

The passive diameter of the vessel, or its maximum capacity of dilation, is reduced. That means that there is a greater than normal rigidity in the vessels, and that they are less able than normal to relax. This symptom is identical to a state of hypertension. But patients with CADASIL display this characteristic even if they do not have hypertension.

Furthermore, in the vessel there is pressure. The myogenic tone allows the vessel to maintain a certain diameter, a certain contraction, in response to the pressure, in order not to dilate passively.

In CADASIL there are two abnormalities. On the one hand the vessel's capacity to relax passively is diminished. On the other hand, for any given pressure the diameter is a bit too wide. Thus, the capacity of the vessel to dilate is diminished.

In the new murine model (mice with the identical physiological condition as a human, but without overexpression of the illness) we can see that the symptom of accumulation of NOTCH3 appears very early (in the first month of the life of a mouse whose normal life span is 24 months). Cerebro-vascular dysfunction is visible at one year, that is, at the adult age of the mouse. On the other hand, the reduced capacity of the vessels to relax becomes apparent at the age of four months.

Question from a conference member: Can the abnormalities of the vessels involve a risk for sportspeople due to the acceleration of their heart rate?

Dr Joutel answered that sporting activity does not pose problems for patients with CADASIL

3. Clinical Research (Professor Chabriat)

Before Professor Chabriat's presentation, Jean-Luc Airiau, President of CADASIL France, gave him a cheque for €10000, payable to ARNEVA (Association for Neurovascular Research at Lariboisière Hospital). This subsidy towards research marks 20 years since the discovery of the *Notch3* mutated gene which is the basis of CADASIL.

Professor Bousser recalled that in 1976, when she was a young clinical head of La Pitié Salpêtrière Hospital, she had met Joseph, one of Jean-Luc Airiau's uncles, who was showing signs of a disease of the small arteries and who had suffered a first stroke even though he wasn't suffering from high blood pressure. The hospital had just recently been equipped with the means to do the first scans.

Professor Bousser had written her thesis in 1972 on the impact of aspirin as a preventative for brain strokes. At that time, aspirin was known for its effect on pain. It was known that it caused bleeding, but it hadn't been considered that it could prevent blood vessels from getting blocked up. Professor Bousser proposed that Joseph should take part in the first major study into aspirin. She had thus followed him up regularly without realising the reasons for his symptoms.

The questions which his family were asked and the symptoms presented by his children over the next few years led to the launching of the research headed by Professor Tournier-Lasserve on the first family. Fifty-seven participants were mobilised thanks to Michel, a family member who contacted all the wider family living in the provinces and told them about it. They all had clinical examinations, MRI scans, blood analyses, etc. This collaboration was essential in order to allow, in 1993, the localisation of chromosome 19 which carries the gene of the illness which was named CADASIL.

In three years, 33 other French families were identified with the same illness and the *Notch3* gene on this chromosome was discovered in 1996.

Professor Bousser appreciates that her former interns have carried on the work after her retirement, and is sure that a way of financing will be found in order to advance this leading research which is internationally recognised.

As for Professor Chabriat, 2016 marks a new stage, 20 years after the discovery of the gene. A therapeutic option has been discovered. Pr. Chabriat's team must prepare themselves for this new stage with Dr Joutel's researchers.

(a) RHU Project

Pr. Chabriat and Dr Joutel have jointly drawn up a plan for a RHU (Hospital – University Research) project centred around CADASIL, including numerous studies (mechanisms of the illness, identification of therapeutic targets, pre-clinical trials, and what is necessary for preparing for clinical trials). The selection of projects will take place at the end of May 2016 with presentations in front of an international jury. Those projects selected by the ANR (National Research Agency) will be financed in the context of a government programme of major investments.

(b) Dysfunction of the vessels in the brain

Having verified, after many different stages, that the antibody anti-NOTCH3 is not toxic and that it can be used in a study on man, it will be necessary to prove its effect on vessels, especially those in the brain.

We must therefore find ways of working which show and measure the dysfunction of the blood vessels in patients who are carriers of this illness. It will also be necessary to show, after a period of taking this tested treatment, that it has a corrective effect.

In the CADASIL mouse, the cerebral flow is increased less by stimulation than in a normal mouse.

And it has been proved in the case of the mouse that the antibody anti-NOTCH3 could correct this abnormality. But will it be possible to produce the same effect in man?

Measuring brain activity in man is done by measuring the electrical activity of the brain with an electro encephalogram. When this activity of the neurons increases in the area of the cortex corresponding to a stimulus, it will lead to a dilation of the blood vessels and an increase in the cerebral flow. What happens is that the neurons need to use more oxygen. This is what is called a neuro vascular coupling.

In man, in order to measure the cerebral blood flow on the surface of the brain and its variation during an activity, we use the technique of functional MRI. For the last two years, volunteer patients have participated in the evaluation of this technique. The volunteer is laid down in the MRI machine, inside which there is a screen showing a moving black and white chess board. At the same time, the person has to open and close his hand. In the part of the cortex responsible for vision and in that responsible for moving the hand, we can thus note a specific variation in the blood flow. This action is repeated several times in a random way for 30 minutes. MRI images are taken in order to acquire the variation of the signal in the different areas of the brain (visual and motor).

At the same time, sensors placed on the person's head allow us to carry out an electro encephalogram.

The aim of this study is to evaluate thirty patients who have the CADASIL mutation and are in the early stages of the illness (before they present any symptoms or a handicap, and before they take any treatment which could modify the results) and thirty people in a control group.

For the moment, results have been obtained from the first 17 patients and control group patients (of the same age and sex). Their average age is 42.

We have established, during both the chessboard visualisations and making the hand movement, that it is these areas of the brain (visual and motor) which are activated and which are subjected to the greatest variation in blood flow.

The size of the area which is activated is identical in both patients and control subjects. During rest periods the blood flow is slightly higher in the control group, but this is not significant in such a small sample.

Another finding is that age does not have an impact on the effect of activation. On the other hand, the length of the stimulus in both motor and visual areas does make a difference, a disparity which is measurable in carriers of the mutated *Notch3* gene.

It remains to be proved that these results are not linked to a reduction in the electrical activity of the neurons, but definitely to an abnormality of the blood vessel.

Given that these facts have been established before people present with symptoms, it should be possible to use this biomarker with this technique in order to measure the effect on the blood vessels of a trial of a preventative treatment. This would be done by looking to see if the disparity observed in the variation of blood flow can be corrected.

This study is being done by a post doctorate, Clément Huneau, with Dr Christian Giroux, who meets up with both the patients and the imaging team.These examinations are very complex, having to combine the electro encephalogram, the collection of all the information on brain activity and the MRI results. If this method proves to be convincing, it will be necessary to find ways to automatize it, because it requires a great deal of human input at the moment.

This research is financed by the Leducq Network and other different institutions. The results will be available in a few months.

Dr Joutel's intervention. From now on, we are trying as far as possible, to reproduce in the patients the findings and the results that we have in the mouse model. There is much close collaboration between the teams of both Dr Joutel and Pr Chabriat.

But the ways of working are different, and more complex to envisage in man, in comparison to a laboratory animal.

We find ways of helping us transpose the analyses, the measurements and the therapeutic paths identified in the mouse model. If a treatment works for a mouse at a very early stage, we must be prepared to prove that it works in an equally relevant way for a man, although his symptoms show themselves much later on.

This is a new direction in our way of working: fundamental research work, which is theoretical, is getting closer to the therapeutic. There is no competition between the teams. Bad experiences with other discoveries, where something has worked in an animal, but has been disappointing in man, will thus be avoided.

In mouse and man we find the same thing, an indicator of the dysfunction of blood vessels in the brain, which is the consequence of an accumulation of the protein.

Dr Joutel and Pr Chabriat have provided evidence to show that in future it will be necessary to take a preventative treatment when the carrier of the mutated gene *Notch3* does not yet display any lesions and is not yet ill. The aim would be to avoid or to slow down the appearance of future symptoms, in the same way as is done in other chronic illnesses like diabetes, hypertension, etc.

Moreover, other trials must be carried out at the same time, using other methods, on patients who are at a more advanced stage, and are already presenting symptoms. For example, another team in Holland is testing a different therapeutic approach which aims to produce NOTCH3 proteins which have no mutation despite the gene being mutated.

The RHU project outlined previously also addresses the necessary question of a preventative treatment and innovative therapy for supporting patients who already have symptoms and are handicapped. It may perhaps not be the case of a single treatment, but of a combination of drugs, according to the different stages of the illness.

All of these studies will require funding.

4. MRI 7 Teslas (Dr Jouvent)

Tests done on volunteer patients who are taking part in the MRI 7 Teslas study led by Dr Eric Jouvent at Neurospin, have been carried out in 2015. Work has been done on this data to understand the microscopic level more precisely.

5. Study of Stem Cells (Dr Joutel)

At the AGM in April 2013, Dr Joutel set out a research project about stem cells. This work has taken place in collaboration with Dr Sanjay Sinha, of Cambridge, England, who is an expert in the

field of obtaining smooth muscular blood vessel cells from Ips cells (Induced Pluripotent Stem Cells). The aim is to cultivate smooth muscular blood vessel cells from the patients who have CADASIL in order to study the reasons why these vessels are the centre of abnormal deposits of NOTCH3 and undergo a process of degeneration. These cells will allow us to understand how the mutated protein clumps together, and to identify if it is the deposits that are toxic or the small accumulations of a few molecules of mutated proteins, etc. The results will be a lot more rapid than when working with mice (that have to be crossed, allowed to grow old, etc.)

Dr Hervé has performed biopsies on eight patients. With these cells, fibroblasts have been prepared and cultured in Dr Joutel's laboratory. They have been reprogrammed as stem cells in England, for five patients who have five different types of mutation, and then redifferentiated into smooth muscular cells.

The first results are very encouraging. It appears that we might see abnormalities such as deposits of NOTCH3.

These cells might constitute another tool with which to test the antibody anti-NOTCH3 and even allow us to improve it.

The Dutch therapeutic strategy, which aims to modify the mutated protein, is also going to be tested on these human cells.

6. Legal obligation to inform the patients' families (Dr Hervé)

A person's obligation to inform his family members if it has been discovered that he has a genetic illness has now been made law in France (in case of diseases for which this information will permit other members of the family to have a preventative treatment or to get genetic counselling).

The National Committee for Ethics expressed reservations about such an obligation.

The objective could well be logical in the cases of early onset rare illnesses and for which there exists a preventative treatment. In the case of CADASIL this obligation could be problematic.

Should the doctor, who has to inform and help his patient, tell him about this obligation in the case of CADASIL?

The necessity of giving support before, during and after the diagnosis to those who want to have a predictive test for CADASIL, justifies the recommendation of CERVCO, the centre of reference, that steps should be taken in the form of a multi-disciplinary consultation.

The experienced practitioners (psychologist, geneticist and neurologist) who carry out these meetings, are better able to give both quality information and to tackle the subject of informing family members before the diagnosis is made, rather than a GP who knows little about this rare illness. Thus, if the person decides to have a predictive test after their initial information gathering consultation, they will be aware of this obligation to inform their family, should the subsequent result prove positive.

But how could, or should the person with a positive diagnosis tackle this subject with those close to him whilst respecting the law.

CERVCO has started to reflect on this need to inform. It is proposed to set up a discussion group, with participants coming from Association members, experts on the illness and the questions it poses, a law specialist, etc., taking into consideration the expression and experiences of patients and their families.

CADASIL FRANCE

Annual General Meeting – April 2nd, 2016

Questions from members and attendees

Question: "In 2015, a Dutch researcher, Dr. Lesnik Oberstein, solicited help on Facebook for research funding. Do you know of her work? More generally, is research on CADASIL primarily conducted by the Leducq network? Which countries are most active besides France? Are there publications and reports about treatment testing in France and abroad? "

Answer: Regarding perspectives on treatment tests, this topic has been covered during today's AGM medical presentations.

Dr. Joutel indicates that the Dutch team did research from a technology (AntiSense Oligonucleotides- ASO) which is also used in clinical trials on Duchenne muscular dystrophy. The research's objective is not to act on the protein itself but rather on the process leading it to be produced. The protein is not the target, which is rather the interaction between the gene and the protein. The approach is to try to remove a small part of what constitutes the code of the mutated protein. This has been validated at the cell level (in vitro). This strategy, if it proves to be effective, is very promising. However, at first, it could be used only to treat patients whose mutation is located in the initial part of the gene, which is the case for about half of the patients. It would prevent the onset of accumulation and should therefore be implemented at a very early stage of the disease.

The Dutch team has generated a new transgenic mouse model of CADASIL with an overexpression of mutated human Notch3 protein. Dr. Joutel was in contact with the Dutch team for consideration in testing the French therapeutic NOTCH3 antibody prototype on this model.

The transatlantic network formed with the support of the Leducq Foundation, which brings together Dr. Joutel 's team and leading laboratories in Europe and the USA, is the most dynamic and advanced network in research on CADASIL.

Question: "Dr. Joutel's team is introduced to us each year. Do they work solely on CADASIL or also on other diseases? In this case, how many people are doing research on the disease? And do some researchers have a temporary contract?"

Answer: Dr. Joutel presented the composition of her team at the end of today's presentation on genetic research. Her laboratory works on other diseases of small vessels in addition to CADASIL. One young post-doctoral member is studying mechanisms of another form of genetic disease of small cerebral vessels.

Research on these other diseases is complementary to that carried out on CADASIL. It helps develop new methods and discover new mechanisms that can benefit research on CADASIL.

The team of Dr. Joutel is also part of a European consortium which aims to identify common mechanisms among various forms of small vessel diseases.

Dr. Joutel's team has three post-doctoral researchers, a part-time engineer working on her thesis, a neurology intern preparing his Master degree and an assistant-engineer. A research engineer recently left the team to relocate to the provinces for family reasons. Outside recruitment via exams, INSERM does not allow staff

recruitment for open-end permanent contracts. Only fixed-term contracts are possible. The maximum duration of these contracts is six years. The three post-doctorate members were recruited on temporary contracts.

Question: "Did the Danish Lundbeck Laboratory file a patent?"

Answer: (Dr. Joutel) This topic was the subject of a presentation during the conference on genetic research today. A patent has been filed indeed by this laboratory in partnership with French INSERM institute. It is about a proof of concept for a treatment strategy, based on antibodies, tested in transgenic mice.

Question: "A young woman in my family who is 35 years old carries the CADASIL gene. She suffered two optic neuritis. It is said to her that this is not related to the disease. But it is not reassuring for her. What is your opinion?"

Answer: (Pr. Chabriat and Dr. Hervé) There is no link between optic neuritis, which is an inflammation of the optic nerve, and CADASIL. It is a manifestation of an inflammatory disease, such as multiple sclerosis, while CADASIL is a vascular disease.

Question: "What is cognitive rehabilitation? Would it work in the recovery in the aftermath of a stroke or delay some symptoms? Who is prescribing and practicing it? Is it covered by Social Security? "

Answer: (Sonia Reyes, psychologist from CERVCO) Cognitive rehabilitation is the process of study and rehab from disorders observed in a particular patient. Depending on nature of disorders (attention, memory, concentration, planning difficulties, etc.), we must try to overcome the deficit gap, on an individual basis, and limit its impact in daily life, according to each person's objectives, taking into account one's situation, age and needs.

Rehab may provide advice to suit the patient's activities, help prepare a request for adjustments in a job position, stimulate remaining function skills, etc. This support does not alter the course of the disease, but it seeks to implement ways to restore confidence, make it easier in daily life and in developing social relations. Some of these actions can be performed with a speech therapist, an occupational therapist, etc.

Question: "How is the disease progressing over time? Nature of disabilities and are there essential treatments? Are there suitable courses of treatments at spas? "

Answer: (Pr. Chabriat) The disease is covered in detail on the websites of CERVCO and CADASIL France. Generally, the first symptoms may be migraine fits with aura. They may appear among 40 to 50% of patients. The severity of the disease is related to the occurrence of successive strokes. These small infarcts will cause various deficit disorders. Their recurrence gradually leads to disabilities such as loss of balance, walking disorders, etc. and retardation.

There is a great variability from person to person as regards to symptoms, age when disorders start to appear and evolution of the disease.

There is no particular thermal health cure that can be recommended. But complementary care including kinesiotherapy, stimulation, proper diet, etc., can be beneficial if it is properly suited to the patient's health and fatigue level.

Question: "Is it good to take vasodilators, i.e. medication to increase blood flow, like Cervoxan which is prescribed for migraine?"

Answer: (Pr. Chabriat) This type of treatment has not been studied for a disease such as CADASIL.

It is therefore not advisable to take such a drug for preventing migraine with aura, because there is no evidence of its effectiveness.

Question: "Since my son will be 18 in May 2016, I strongly wish that he makes a decision to do genetic blood testing (he told me about it recently). Can I perform it here on premise? Since we live far from Paris, travel is difficult. How should I proceed in this case? I would appreciate kindly if you can give the exact description for the blood test so that my general practitioner could prescribe and forward it. "

Answer: (Pr. Chabriat and Dr. Joutel) A parent shouldn't ask for this type of genetic testing on behalf of his adult son. It is up to the son to contact directly the CERVCO referral center, in order to consider scheduling a multidisciplinary appointment so that he can be assisted in his decision in an informed manner. He can also make an appointment with the geneticist physician of the hospital in his region. The CERVCO may provide contact information of these geneticists in the provinces.

Question: "Dr. Joutel talks about future medical trials. This hope of reaching a cure one day (and everyone is anxious for it) is what supports us. We have difficulties in imagining different stages to obtain the approval for a drug (or treatment). We hear about evaluation phases 1, 2, 3, etc. Could you tell us where we are today and what steps have already been accomplished? What steps are left to go? What are their potential durations? Can we have an idea of the process costs and how we can hope enough funding will be found? "

Answer: (Dr. Joutel and Pr. Chabriat) The initial step, prior to phase 1, in evaluating an hypothesis of treatment strategy is to evaluate the potential drug on animals. This process includes verifying its effectiveness, defining mechanisms of action of the treatment, determining biomarkers as proof-of-concept and tools permitting to measure and monitor the effect of the tested drug.

It is also necessary to evaluate the toxicity of the treatment, generally in two different animal species. For these toxicity tests, the drug must be produced in sufficiently large quantities under very controlled conditions. Such a stage would last about five years. If we would manage to pass this animal stage with antibodies testing, the clinical phase could be faster compared to other drugs because there are already a lot of data on this component.

In humans, phase 1 lasts about a year and a half. We first study reactions in healthy subjects.

Phase 2's goal is to assess dosage of the drug as well as potential tolerance and toxicity. The group of people participating in the test receives different dosages in order to identify which one is the most effective with no side effects.

Phase 3 is used to test effectiveness based on clinical criteria or markers of effect assessment e.g. MRI.

These last two steps require a few years, in general, based on number of participants.

For a test to have high probability of success, we must be able to prove it: we must take precautions to properly select the composition of the treatment sample, select participants at an age and stage of disease corresponding well to the target mechanism of the treatment, have identified and validated markers so that the effect can be validated and measured.

It is better not to rush in order to make sure that the strategy is good in humans.

In the case of the NOTCH3 antibodies strategy currently under CADASIL evaluation, it is very important to validate its effectiveness in several mouse models, and to try developing evaluation methods transposable to humans, in order to minimize the risk of failure. It is also important to understand the mechanism of action of the treatment and to have established a method to measure its impact.

It will cost several million euros to arrive at a treatment. The support of an association is critical to collect donations and grants.

Projects on therapeutic tools to prevent or delay effects of CADASIL disease are complemented by other research work on finding ways to care for and improve conditions of patients who are already experiencing problems related to the disease.

An anti-tobacco campaign is also needed, since smoking triples the risk of stroke!

A Chinese post-doctorate researcher has just joined Pr. Chabriat 's team in a program of cooperation between the Chinese and French Academies of Sciences, as well as thanks to a grant from ARNEVA (Association for Neuro-Vascular Research at Lariboisière Hospital). He will lead a study on blood pressure of arteries. We will study its role in the disease and the occurrence of new small infarcts, using MRI. It might be possible to intervene early enough if we could evaluate pressure variation indexes that are associated with the occurrence of strokes.

It is also important to help patients recover from a succession of strokes and improve rehab of functional state. CERVCO also supports projects in this area.

Question: "This year, I came across an article about chromosome 19 which says that there may be mutations following vaccinations. As I recalled, they were talking about polio and whooping cough (pertussis). I would like to get your opinion."

Answer: (Dr. Joutel and Pr. Chabriat) This article is not scientifically possible. Infections can not cause a mutation responsible for this disease. Information on the Internet should be interpreted with great caution and one must refer to reliable sources. If in doubt, you should inquire with your physician or CERVCO.
