CADASIL France

Annual General Meeting March 21st 2015

Presentations of the Medical Team

At the Annual General Meeting of CADASIL France, many members of the medical team attended the afternoon session which was given over to medical presentations: Professor Hugues CHABRIAT (Head of the Neurology Department at Lariboisière Hospital, Co-ordinator of CERVCO, Centre de Référence des maladies Vasculaires rares du Cerveau et de l'Œil); Dr Anne JOUTEL (Research Director at INSERM, responsible for biological and genetic research into CADASIL); Mrs Annie KURTZ (Psychologist in the Department of Neurology at Lariboisière, and a committee member of CADASIL France); Dr Dominique Hervé (hospital practitioner, Neurologist, responsible for CERVCO); Dr Eric JOUVENT (University Lecturer and a practitioner at Lariboisière); Mrs Christina ROGAN, Sonia REYES and Aude JABOULEY (Psychologists at CERVCO); Mrs Jocelyne RUFFIE (Assistant to Professors BOUSSER and CHABRIAT); Mr Abbas TALEB (Clinical Research Assistant); Mrs Stéphanie MOREL (Social Assistant at CERVCO); Mr Clément HUNEAU (Post Doctoral student); Dr Christian GIROUX (Neurologist, assistant hospital practitioner working in the Neurology Department of Lariboisière).

1. The Clinical Research (Professor Chabriat)

Professor Chabriat's presentation focused on a study which will be the subject of a forthcoming publication. It allowed the identification of the clinical elements and aspects of the MRI scans which enable the prediction of the illness over a period of three years. The precise factors which have been established will be able to be used as criteria for evaluating the effectiveness of future trials of treatments.

A. A study of the predictors of clinical decline in CADASIL. A prospective study of 290 patients followed for three years.

To be included in this study, the patients had to be carriers of the mutated NOTCH3 gene.
The study was carried out with 290 patients, of whom about half were followed at CERVCO and the other half in Germany at Munich.
Some of the participants left the study during the three year period (for different reasons: too long a delay in coming to an intermediary appointment, a worsening of the illness preventing travel to Paris hospital, death, etc.). Some of them were contacted at home by the CERVCO team in order to obtain information about the evolution of their illness, but that did not allow all the parameters to be collected.
In total, 236 participants made up the cohort, whose average age was 50.

Much data was collected from each of the participants, with the same criteria and the same intervals between two evaluations. Their consent was obtained.
It was necessary to have resources to build up a database, which progressively gathered together the information about each participant over three years. This database was handled by a specialist company, Orgamétrie, which needed financing.

During the first visit, questions were asked on clinical aspects: had the patient suffered a transient ischemic attack, or a stroke (a cerebral attack characterised by the trace of a small infarct on the MRI scan), and/or an epileptic fit, did he have problems with walking, memory, attention, concentration, irritability or depression, what was his age, his level of education (this has an impact on the tests), did he have other risk factors (diabetes, high blood pressure, high cholesterol levels, smoking, alcohol, etc). Examinations were also done (blood tests and measurement of cholesterol levels).
As well as this, the evaluation of each patient included the measure of their handicap according to a scale (Rankin: 0 and 1: no handicap; 2: a small problem; 3: needing help at particular times; 4: needing help in their everyday life; 5: significant handicap).
In addition, tests were done with a psychologist to establish a MMS score (Mini Mental Score).
During the MRI scan, we measured the anatomy of the brain (its volume related to the size of the inter-cranial cavity), we looked for lacunae (small localised areas of dead tissue showing up as spaces), we counted these and checked their volume, we looked for micro-bleeds (showing up as “tattoos”, small marks linked to the iron from the blood corpuscles which have leaked out of the vessels).

The cohort was made up of 55% women.
19% of the participants had high blood pressure, 40% had high levels of cholesterol, 30% had problems with walking, and 30% had problems with balance, 40% said they had suffered migraines with aura, 65% had suffered a transient ischemic attack or a stroke, 18% had either a moderate or severe handicap, 13.5% had a cognitive deficiency which had an effect on their daily life.
MRI scans showed that the participants had on average 5 lacunae (small cavities in their brains), 1 out of 3 had micro-bleeds, and the average cerebral volume in relation to the capacity of the cranium was 85%.

The results which were collected from all participants at the beginning, middle and end of the three year period, were analysed and showed that:

- If a patient presents with walking difficulties, the risk of having a stroke after a period of 3 years is multiplied by 4.6
- If he has already had a stroke, the risk of another stroke is multiplied by 4
- If he smokes, the risk of a stroke is multiplied by 2.2
- The risk of a disability is increased in people who have cognitive deficiencies or problems with walking

- The greater the degree of disability, the greater the risk of a cognitive decline (if their Rankin score is 3, the risk is increased by 25%)
- Smoking triples the risk of cognitive decline
- Being male increases the risk of developing a serious form of the illness much earlier

From a clinical perspective we can therefore say that problems with walking, smoking and having had strokes are the prognostic indicators of CADASIL.

From an analysis of MRI images over three years, the following results have been obtained:

- The risk of having a stroke is increased in people who have more than 3 lacunae (infarcts) or micro-bleeds
- The onset of disability correlates to the initial volume of the brain
- The cognitive deficiency could be associated with the cerebral volume and with micro-bleeds

To sum up, in MRI scans, the factors associated with having a stroke or a cognitive decline or a disability are: the presence of more than three lacunae as seen on an MRI scan, a reduced cerebral volume and the number of micro-bleeds.

These markers from a neurological examination and MRI scan have therefore to be taken into account when predicting the evolution of the illness in a patient over time.

In conclusion, in patients who have CADASIL and are being followed over three years:

- The occurrence of a stroke in the past is associated with a greater risk of a stroke in the three years of the study (4 times greater than those who have not had one)
- The appearance of a new stroke during the three year study is not significantly associated with a cognitive decline (certain patients recuperate very quickly)
- The presence of either a moderate or severe disability is a predictor of a worsening clinical condition during the three years
- Regular smoking doubles the risk of a stroke and triples that of cognitive decline over three years
- Patients whose MRI scans show more than three lacunae have a 5 times greater risk of having a stroke
- Reduced cerebral volume is a predicting factor in clinical evolution

All this information will be essential in the preparation of the therapeutic trials of tomorrow.

The cost of this study is estimated at about €1,000,000. This budget has been financed thanks to past ministerial funds, (two “Programmes Hospitaliers de Recherche Clinique”) and to subsidies received from ARNEVA (Association for Neurovascular Research at Lariboisière Hospital), and to CADASIL France, which for several years has partly financed the salary of a

Clinical Research Assistant etc, without counting the investment in time and the motivation of the CERVCO team.

B. Number of subjects required for a study of a treatment.

Thanks to a knowledge of the factors which predict the clinical evolution of the illness, mathematical calculations allow us to determine both the criteria for evaluating the effectiveness of treatments and the number of people necessary to participate in the evaluation of a treatment.

So, if one wanted to measure the efficiency of a treatment according to its impact on the arrival of an unexpected stroke, in order to reduce the risk over three years from 20% down to 10%, 394 participants would be needed.
If one takes into consideration the fact of having a stroke, or a decline in cognitive abilities, or a small handicap, the probability of one of these happening is 41% over three years. 144 participants would allow us to prove the efficiency of a treatment which would halve the risk.
The objective is to explore all the possible combinations coming from our existing data, and to use them to prepare a therapeutic trial which is feasible and which does not cost too much, by involving a number of participants which is not too big, but sufficient to prove its effectiveness.
The database will always be fed from the monitoring of patients (some since 2003), although there is no longer specific financing to do so. The knowledge will therefore continue to grow.

A delay of some years is still necessary before we envisage the evaluation of the treatment in man. Financial and human resources are also needed.
We need to think about treatments which work on the development of symptoms, but equally about innovative strategies which could have more impact. We also need to consider parameters which have not stood out from the predictive factors. In particular, blood pressure measurements are taken regularly during follow-up consultations, when in fact noting the variation of blood pressure over a 24 hour period, even when it is apparently normal, could be just as interesting.

Already, a preventative step is being taken when aspirin is being prescribed to patients for empirical reasons. Generally speaking, we know that the formation of a blood clot often leads to a stroke. Aspirin and Kardegic are anti-clotting agents which lessen the risk of the formation of small clumps of platelets which block the vessels. Thus, they are generally recommended if an infarct is seen on an MRI scan. But there is no proof that this is really effective in the case of CADASIL.
To be precise, an infarct is a lesion in the brain, an area having suffered damage, which causes a stroke. Depending on where it is situated, an infarct can be “silent” and not cause any symptoms.

2. The Genetic Research (Doctor Joutel)

During the past year, Dr. Joutel has continued her research work with her team, prior to the clinical research. The aim is to understand the mechanisms of the illness, in order to try and develop therapeutic strategies in collaboration with Professor Chabriat's team.

A carrier of the mutated gene NOTCH3 from birth will in time develop lesions in the vessels and in the brain. Initially, these lesions will not cause any bother and will have no impact on daily life. It is a case of an accumulation of NOTCH 3 protein in the vessels which can be detected by a skin biopsy, and of hyper signals in the white matter (white patches), in fact small cerebral infarcts, which are visible on a brain MRI. Such a person who is a carrier of a mutation of the NOTCH3 gene is called "asymptomatic" because he does not display any clinical symptoms.

With the evolution of the illness, clinical problems appear, leading to difficulties or even a greater handicap. The illness CADASIL is then perceived by the patient who is said to be "symptomatic".

Several strategies for treatment can be envisaged.

Very schematically, a treatment given at the asymptomatic stage of the illness will have as its goal preventing or slowing down the progression of lesions of the vessels and of the brain, and thereby preventing or slowing down the appearance of the clinical symptoms and of a handicap. This type of treatment can be qualified as preventative because it acts before the person experiences the effect of the illness. A treatment given at the symptomatic stage of the illness will aim, as far as it can, to attenuate the handicap or the troubles caused by the lesions in the brain.

The CADASIL mouse models which are currently being used reproduce the initial stages of the illness. The experimental studies based on these models try to understand the mechanisms behind the apparition of these lesions in the blood vessels and in the brain. They will lead, in all likelihood, to preventative therapies. It is equally important to research treatments to lessen or treat the symptoms which patients find more troublesome. But the means and the experimental models for such research are at the moment very limited. In particular, there are no mouse models which mimic the advanced stages of the illness.

The application to humans of preventative therapies which have been effective in mice, remains problematical with illnesses such as CADASIL. It poses the question of how to evaluate the efficacy of a drug given to people who do not yet display any symptoms. In actual fact, the pharmaceutical industry has to provide a proof of efficiency to obtain an authorization to market the product ("Autorisation de Mise sur le Marché"). It also poses questions of an ethical nature: asymptomatic people would be advised to perform a genetic test so that, in case the result shows they

carry the mutated Notch3 gene, a preventative treatment would be given to them, in order to prevent possible symptoms they might present many years later…

In the last year, progress has been made in several areas:

A. Mechanisms of Cerebro-Vascular Dysfunction

The CADASIL mouse model, which has been used since 2010 and carries the mutation of a patient, overexpresses the protein NOTCH3. With age, these mice develop deposits of NOTCH3 in their blood vessels, deposits of GOM (Granular Osmiophilic Material), a dysfunction of the cerebral blood vessels, then lesions of the white matter. Other laboratories in Europe or the USA are carrying out research using this model. The proliferation of work throughout the world using these mice will allow the development of new ideas and the exploration of new paths.

Thanks to a technique brought over from the USA, a researcher (Carmen Capone) has studied the reactivity of the cerebral blood vessels in these mice. Using a living anaesthetised mouse, a small hole is made in the cranium, and a laser probe (Doppler Flow) is positioned on the brain to record the variations in the cerebral blood flow in response to different stimuli. This hole also allows pharmacological substances, proteins, antibodies, etc. to be administered. This technique has allowed us to list in detail the functional anomalies of the cerebral blood vessels of CADASIL mice.

Alongside this, the mechanical properties of small arteries on the surface of the brain are being studied. These arteries (with a diameter of about 150 – 160 microns) taken from dead mice, are being dissected under a microscope. We know that one characteristic of these small arteries is to contract in response to a raising of “intraluminal” pressure by developing a “myogenic tone”. Studies done in the laboratory show that the myogenic tone of the cerebral arteries is very changed in the CADASIL mouse.

In the United States, Dr Mark Nelson’s laboratory, which has been collaborating with the French team for three years, is able to dissect the arteries from within the brain. It is a question of the extremely small vessels, with a diameter of about 20 microns. CADASIL mice have been transferred to this American laboratory. Mark Nelson’s laboratory showed that the myogenic tone of these arteries from inside the brain was also very altered. Céline Baron-Menguy, medical engineer in Dr Joutel’s team, went to spend a week in Dr Nelson’s laboratory, in order to learn this latest advanced technique.

Anotherprocess is practised by Dr Nelson’s team: the smooth muscle cells from the wall of the cerebral vessels (those where the mutated protein plays a role) are isolated and ionic currents (structures on the surface of the cell which allow ions like Potassium, Chlorine and Calcium to pass through) are recorded. Dr Nelson’s laboratory has shown that the reduction of the myogenic tone in the arteries of CADASIL mice is caused by a hyperactivity of the potassic canals at the level of the smooth muscle cells.

Recently, Dr Joutel's laboratory has identified in the GOM some proteins which accumulate abnormally as a consequence of the accumulation of the mutated protein NOTCH3: TIMP3, vitronectin, and several other proteins. CADASIL mice have been crossed with mice which express half the amount of TIMP3 or none at all, a reduced quantity of vitronectin or none at all. Mice which overexpress TIMP3 have also been created. These different types of mice have been left to grow old, while the evolution of their illness has been followed.

Thanks to this type of experiment, the laboratory has shown that the abnormal accumulation of the protein TIMP3 is responsible for cerebro-vascular dysfunction.

Therefore, we are beginning to understand the mechanisms of the illness at the level of the blood vessels, then of the smooth muscle cells and the canals of the cell itself, and we understand in quite a precise way, the mechanism which leads from the mutation to the dysfunction of the vessels.

We have learnt from these approaches that the mutation of the NOTCH3 gene leads to the accumulation of NOTCH3 protein, which itself leads to an increase in the quantity of TIMP3 protein, and to a whole succession of other events. This string of events finishes by increasing the expression from the potassic canals on the surface of the smooth cell walls which in turn goes on to lessen the tone of the blood vessel and leads to its dysfunction.

B. A note concerning the mechanism of the lesions of white matter in their initial stage.

An advance was made during the previous year by a neurologist, Emmanuel Cognat, who was working on his thesis in Dr Joutel's team. He had worked on the characteristics of the lesions of the white matter in a CADASIL mouse, and found a marker which allowed him to quantify them.

The laboratory continues to explore the exact significance of lesions of the white matter which have been observed in CADASIL mice. In particular, using MRI scans, the appearance of these lesions will be compared with that of lesions which have been observed in patients. This study is led by Professor Chabriat and Dr Jouvent.

The laboratory has discovered that the abnormal accumulation of vitronectin contributes significantly to the lesions of white matter.

In fact, when a CADASIL mouse is crossed with a mouse which no longer expresses vitronectin, or only a reduced amount, we are able to very significantly attenuate the lesions of white matter.

As previously indicated, the accumulation of TIMP3 impacts on cerebro-vascular dysfunction. On the other hand, it does not seem to make a difference to the lesions of white matter: if one interferes with the quantity of TIMP3, that does not change the severity of the lesions of white matter.

Work done already suggests the following mechanism in the initial stage of the illness: the mutation of the gene NOTCH3 leads to an excess of this protein

NOTCH3, which modifies the expression of a certain number of other proteins around the blood vessel, like TIMP3 and vitronectin and other proteins. Each of these proteins seems to contribute to how the illness is expressed. The excess of TIMP3 is responsible for the dysfunction of the cerebral blood vessels and vitronectin is responsible for the appearance of lesions of white matter. Other proteins which are currently being investigated are probably also linked to certain symptoms.

There are also some other questions. Notably, the fundamental characteristic of the illness is the formation of deposits of NOTCH3. Is it these deposits which are toxic? Even before the formation of these deposits, perhaps it is the accumulation of the first molecules (small packets) of the NOTCH3 proteins which generates a cascade of events… These matters must be studied to decide whether the deposits should be eliminated by some sort of treatment.

C A note on pre-clinical trials in CADASIL

One compound, identified jointly by both a Danish pharmaceutical laboratory and Dr Joutel's laboratory, has been tested on the CADASIL mouse model. Dr Joutel's laboratory has demonstrated that this compound completely corrects the dysfunction of the small blood vessels in the brain of this mouse model!

It is not a question of testing a drug, but of proving a concept.

A patent application has been requested by this pharmaceutical laboratory and Inserm. There is a delay of at least a year before it will be in the public domain, which will allow the work to be completed. Then, the company can decide whether or not to invest in continuing these studies. This finance is indispensable in order to envisage a future clinical trial in man.

This moderately-sized company (about 5 000 employees), which specializes in developing drugs for neurological illnesses, only launches clinical trials in man if there is a strong chance that they will be effective. The objective would be to launch a clinical trial within five years.

But a lot of different work is still necessary before contemplating a clinical trial in man.

In particular, it would be desirable to replicate the results already obtained in another CADASIL mouse model (which is not overexpressing the mutated protein). A pre-requisite of this work would be to use this new model of mouse with these characteristics, which has been created by a Belgian team. It is even possible that this compound turns out to be more efficient in mice that do not overproduce the mutated protein. In addition, even if the use of this compound has come about from a relevant idea, the exact way it works remains to be determined. This stage is very important in the production of the final drug. Finally, its toxicity must be studied. The day when a drug trial will be envisaged, we must decide to whom it will be offered

(young people before they have any symptoms, and/or patients at a greater or lesser advanced stage in the evolution of their illness), the number of participants, the criteria for evaluating its efficacy, etc.

D A note on the iPS Project (Induced Pluripotent Stem Cells): obtaining a cellular model of CADASIL

At the AGM of April 13th 2013, Dr Joutel laid out the details of a research project on stem cells.

This work has been started in collaboration with Dr Sanjay Sinha from Cambridge, England, who is an expert in obtaining vascular smooth muscle cells from iPS cells (Induced Pluripotent Stem Cells).

To obtain these cells, a skin biopsy was taken by Dr Hervé from five volunteer patients who were being followed by CERVCO, in order to isolate fibroblasts and then to cultivate them. These cells carry the genome of the person, with the CADASIL mutation of the NOTCH3 gene. They were sent to Cambridge where the fibroblasts were transformed into “cells which do everything”, called induced pluripotent stem cells. Their reprogramming into vascular smooth muscle cells is in progress.

The objective is to cultivate vascular smooth muscle cells from patients who have CADASIL in order to study the reasons why these cells are the site of the abnormal deposits of NOTCH3 and why they undergo a process of degeneration. They will allow us to understand how the mutated protein builds up, and to identify if it is the deposits which are toxic or the small packets of a few molecules of mutated protein. The results will be much more rapid than working with mice (that have to be cross-bred, and grow old, etc.)

Dr Joutel’s team is made up of three post-doctoral researchers; Lamia Ghezali, who is working on a pre-clinical study, Carmen Capone, who is studying vascular dysfunction, and Julien Ratelade, who is working on the mechanisms of the lesions of white matter, and on another type of disease of the small arteries, which will provide some useful information for CADASIL. Céline Baron-Menguy, research engineer, and Véronique Domenga-Denier, assistant engineer, complete the laboratory team.

Also, an American-Danish laboratory is currently collaborating with Dr Joutel in the study of glymphatic clearance, that is to say the movements of fluid surrounding the blood vessels in CADASIL.

The contribution of new researchers and the studies done in different laboratories around the world are creating new approaches, ideas, methods, techniques and knowledge. This is indispensable for making more progress. One of the many challenges to overcome is the creating of a mouse model with mutations replicating the advanced stages of the illness, that is to say, mice which would develop cerebral infarcts, handicaps and cognitive problems.

3. MRI 7 Teslas (Dr Jouvent)

Working with the cohort of patients who are being followed at Paris and Munich, analyses have allowed us to identify different types of hyper signals from the white matter during their cerebral imagery. Certain hyper signals which are found under the cortex in the temporal and sub-cortical regions (more superficial) do not seem to have the same impact as the hyper signals found around the ventricles (much deeper).

Therefore at the moment, analyses are being done on a few volunteer patients who have taken part in the 7 Teslas MRI Study, led by Dr Eric Jouvent at Neurospin, to determine if it is effectively possible to distinguish different types of lesions in the white matter.

One hypothesis to explain the appearance of hyper signals is tissue damage in the deeper regions of the brain. But that is not completely satisfactory. Actually, some patients have more white patches than others who have a larger brain.

This could be caused by an inter myelenic oedema (myelin is the sheath around the neurons), which is the origin of an increase in water in the white matter which appears swollen (oedema of the white matter).

4. M. Clément Huneau - Post-Doctoral Researcher

If a future drug is destined for young adults who are carriers of the mutated gene, with the aim of preventing the illness developing some tens of years later, how are we going to verify if it is efficient?

New methods of evaluation are necessary to find an eventual dysfunction well before the first symptoms of the illness, and to identify if this new measure can be used to quantify the effect of the treatment.

Thus, at the outset of the illness, we have observed in mice lesions and oedema in the white matter. Can this oedema also be seen in man at the beginning of his illness? Can it be measured, or can its evolution be followed to determine the effect of the treatment? This area is being studied by Dr Jouvent, using MRI 7 Teslas.

What is more, Clément Huneau, medical engineer, is currently exploring neurovascular dysfunction by using new methods of cerebral imagery. This study is being supervised by Professor Chabriat.

It is a question of evaluating the neuron/blood vessel link in CADASIL.

This project is being made possible thanks to funding by the Leducq network, an American foundation.

Whenever we make a movement, that activates the neurons in an area in the corresponding hemisphere of the brain. The neurons need energy in order to

function, that is to say, glucose and oxygen, which are carried by the blood vessels surrounding the neurons. The neurons demand energy by increasing the rate of blood flow locally and temporarily.

The mechanism is the following: the activity of the neurons, which is caused by a stimulus, is captured by the contractile cells in the heart of the blood vessels, so that they can dilate and contract.

As it happens, the NOTCH3 protein is found on the contractile cells….

In order to find out if the link between the rate of blood flow and the activity of the neurons is altered in people who carry the mutatedNOTCH3 gene, at an early stage, the following examination is done:

- People participating in this study have an MRI scan during the course of which various stimuli (visual and motor) are applied.
- An electro-encephalogram measures their neuronal activity.
- The MRI scan allows the rate of blood flow to be measured.

This study has been carried out on 60 people, 30 of whom carry the mutated gene. Ten participants have already done this test, which lasts around two hours. The first results are very encouraging.

Mathematical models will be used to understand the links between the signals from the MRI and the electrical signals from the encephalogram, and to compare them with the results of the 30 people in the control group (who have not got the illness), and the 30 carriers of the mutated NOTCH3 gene.