GENERAL ASSEMBLY

CADASIL FRANCE - March 31, 2012

PRESENTATIONS AND CONTRIBUTIONS OF MEDICAL TEAM

Many members of the medical team attended the "Cadasil France Remembrance Day":

- Professor Marie-Germaine BOUSSER (Cadasil France - Scientific Board President),
- Professor Elisabeth TOURNIER-LASSERVE (INSERM Research Unit Director and Head of CERVCO Genetics Laboratory),
- Professor Hugues CHABRIAT (Head of Neurology Department, Fernand Widal and Lariboisière Hospital Group, CERVCO Coordinator),
- Doctor Anne JOUTEL (Director of Research at INSERM, in charge of genetic research on CADASIL),
- Mrs. Annie KURTZ (Neurology Department psychologist of Hospital Lariboisière and member of Cadasil France's Administrative Council),
- Doctor Dominique HERVE (hospital doctor, CERVCO neurologist in charge),
- Mrs. Sonia REYES and Mrs. Aude JABOULEY (psychologists at CERVCO),
- Mrs. Jocelyne RUFFIE (Professor Bousser's Assistant),
- Mrs. Solange HELLO (CERVCO secretary),
- Mr. Abbas TALER (Clinical Research Associate),
- And Mrs. Yao Ming (Chinese neurologist of Beijing Hospital working with CERVCO Team).

Meeting Agenda:

1 - Genetic Research
2 - Clinical Research; Activities and Projects of CERVCO; Hospital Clinical Research Protocol
3 - Questions and Answers

1 - Presentation on Genetic Research

Dr. Anne Joutel outlined recent advances, which were results of work accumulated over many years. The journey was strewn with scientific and technical difficulties. It has led to a better understanding of the disease, especially what happens at the level of Notch3 gene receptor when it was mutated and in the cell.

As a reminder, last year's presentation had focused on the belief that CADASIL mutations give the Notch3 protein a new function. To decrypt it, Dr. Joutel and her team had gone from the observation that regardless of the Notch3 gene mutation, - and there are over a hundred different mutations of this gene, depending on the family- there is always an abnormal accumulation of Notch3 on the surface of smooth vascular muscle cells and deposits of GOM (Granular Material Osmiophilic) seen under electronic microscopy. Moreover, the work of a Japanese laboratory and her laboratory had revealed that GOM deposits contain indeed Notch3 protein.

Based on these observations, she had advanced the hypothesis that Notch3, by accumulating inside in the GOM, would act as the "glue" for other proteins and sequestration of these proteins within the GOM would be the root cause of the disease. Specifically, the abnormal accumulation of these proteins around vascular cells could be toxic and cause of the degeneration or dysfunction of vascular cells. Or the "conversion" of these proteins in the GOM could result in a reduction in their normal functionality.

It was shown that one of these proteins, called here CAD1, accumulated abnormally in brain vessels and in the GOM among CADASIL patients. The extracellular domain of Notch3 interacted with CAD1 and a significant amount of Notch3, as seen in CADASIL, created favorable conditions for the aggregation of CAD1.

Study of mice carrying CADASIL gene supported the hypothesis of a reduction of activity of CAD1 protein, which is sequestered in the GOM.

Since the 2011 General Assembly, research continued on increasing knowledge of the composition of GOM and the identification of these proteins.

Proteins aggregated in the GOM were identified by fractionation techniques on arteries of mice carrying a mutated Notch3 protein. We begin to understand how GOM are manufactured and why some proteins are attracted to them. One of these identified proteins is named TIMP3 (Tissue Inhibitor Metalloproteinase 3). It was found that it accumulates in arteries of mice with a Cadasil mutation, starting early, and autopsy analysis of brains from Cadasil patients showed a significant increase (thirty times above normal) of this protein in the vessel walls. Regardless of whether or not there are mutations in the Notch3 gene, TIMP3 interacts with Notch3 proteins. But when these accumulate in the GOM, resulting in more TIMP3 proteins being stuck on the extracellular domain of Notch3, the interaction of these two proteins is amplified.

Another protein, Vitronectin, interacts very little with Notch3. On the other hand, it interacts with TIMP3, which led to its dysfunction when it accumulates in the GOM.

The results suggest the following scenario. Initially, in a normal cell, there is a little bit of Notch3, TIMP3 and Vitronectin. When the Notch3 receptor is mutated, it accumulates abnormally, so he recruits TIMP3, which in turn attracts Vitronectin and probably other proteins. Some of these proteins have been identified, others remain to be determined. Establishing a complete catalog of these proteins would require a full research project. The accumulation of Notch3 leads to the manufacturing of GOM after a certain time.

Does the accumulation of TIMP3 and Vitronectin explain the vascular lesions?
It turns out that TIMP3 plays an important role in the renewal of the extracellular matrix (the "gel" which wraps around cells). Moreover, work of the research team indicates that TIMP3 plays an important role in the functioning of brain arteries and capillaries.

TIMP3 sequestration could reduce its normal activity. Conversely, the interaction between TIMP3 and Notch3 could stabilize TIMP3 and increase its activity. To explore these hypotheses, the team obtained from other laboratories mice carrying a reduced amount of TIMP3 and cross-bred those with transgenic mice carrying mutated Notch3 protein ("Cadasil mice"). The objective is to determine whether the reduction of TIMP3 in CADASIL mice worsens or improves lesions. Experiments are underway but have been delayed by technical difficulties. Vitronectin is also functionally important for the extracellular matrix. The team uses a similar approach to test its involvement in disease.

Research has been continuing to focus in the effect of mutations on the activity of Notch3 receptor in the context of cerebral arteries.

The Notch3 gene code is for the manufacture of a receiver, i.e. a protein that sends a signal. So far, only the trail of a decreased activity of Notch3 had been explored, with conflicting results between mutations and between studies. In particular, when the team's research had shown that some mutations did not diminish the activity, the work of a group of American researchers led to believe that mutations caused a still lower receptor activity. Nevertheless, this U.S. study was flawed. If we analyze the mutated Notch3 protein alone, a decrease of receptor activity may be the case. But in reality, patients also have normal Notch3 proteins in addition to mutated proteins and their coexistence must be taken into account. A student from the team has conducted a research that led to a thesis. He has developed very sensitive markers to measure precisely the level of activity of Notch3 in cerebral arteries. He had used it for different mouse models, with activation or inactivation of Notch3. Then he studied these markers in the cerebral arteries of both types of Cadasil mice. His results suggest that the mutated protein in the presence of the normal protein is responsible for an

increase in activity of the receptor Notch3. These results need to be confirmed in the cerebral arteries of patients.

This work was complemented by a U.S. team working with Dr. Joutel's team. Professor Mark Nelson of the University of Vermont and his team have used their expertise to analyze the contractility of small arteries inside the brain. In France, we can only study arteries on the surface of the vessel. In mice carrying the mutant Cadasil gene, he demonstrated that there was a more important anomaly on intra-cerebral arteries and there was a malfunction of some channels under control of Notch3.

Another advance is the collaboration with a Danish laboratory (Lundbeck). In partnership with this laboratory, the team will conduct a therapeutic study on CADASIL mice. This is a proof of concept which aims to test a solution to remove Notch3 deposits or prevent its appearance. This study will take thirty months. This Danish company is specialized in the treatment of neurological diseases. It is investing in the research, which proves that the company has confidence in this study.

Dr. Joutel is assisted by a team of six young researchers, bringing together a spectrum of diverse skills and working on different aspects of research: protein markers of activity, vascular contractility.

Professor Bousser emphasizes the importance of the work of Dr. Joutel and international collaborations (Mark Nelson in the U.S. – a "super specialist in the neurovascular field, with expertise in strokes (AVC), who discovers the impact value of his work on diseases of small vessels "- the German team of Prof. Dichgans, another U.S. team studying diseases of small vessels, a team of genetic epidemiologists from Bordeaux, etc..)

Cadasil is increasingly discussed in scientific conferences; interest among scientific circles is growing more and more.

A Franco-American research was submitted by Dr. Joutel (with Mark Nelson) to a foundation with substantial resources, the Leducq Foundation (founder of Ellis Laundry Group). This year, 100 projects were submitted, 12 were selected initially, before a selection of final four who will receive funding.

(Note: After the General Meeting, the project presented by Dr. Joutel was selected as finalist with three others, which is great encouraging news for researchers, confirming the seriousness accorded to study of Cadasil, and for families affected by the disease).

2 - Clinical Research - Follow-up Study of Patients and Activities of CERVCO

Regarding the follow-up study of patients, which began nine years ago, 250 Cadasil-affected people were monitored, some for more than five years, others three, etc.. The same study was conducted on 150 to 200 patients in Germany, with the same tests over a long period, using the same methods and with the same data analysis. This collection of clinical information, imaging, and symptoms aims to better understand the disease and its evolutionary factors. It makes possible the optimization of resources needed for a future clinical trial, to establish criteria of efficiency, number of participants and test duration.

Many data were collected as part of the monitoring protocol. They require substantial analysis work and quality control. This study protocol has ended and has no more funding. But the database must be supplemented by information collected during patient visits that are still being followed by CERVCO.

Mr. Abbas TALER, clinical research associate, is responsible of the task of completing and certification of this database. Cadasil France made a grant of 10,000 euros to help finance his position.

It is still yet left to "monitor" the data (reliability, quality, missing information) coming from approximately 170 consultations carried out at CERVCO.

The results will be published in the near future.

Overall, during the follow-up period, less than 20% of patients experienced a significant clinical worsening after two or three years. We must therefore extract the elements that determine the risk factors of evolution, and selection criteria (e.g. evolution is a little slower among women) useful for a therapeutic test.

Another aspect of data analysis, in addition to the clinical course, is to identify markers of disease under imaging.

In a therapeutic trial, except clinical events such as strokes, etc.., abnormalities seen on MRI (white matter lesions, small infarcts of the brain, brain volume ...) can be measured.

Dr. Yao Ming, Chinese neurologist, currently deployed at Lariboisière Hospital, is working on the imaging data, the link between brain size and the lesions observed on MRI.

In the past, the volume of white spots was not considered as a predictor sign of the development, severity of illness and disability.

It was also shown that small infarcts are the main markers of severity and disease progression. They are associated with a greater reduction in brain volume.

Mrs. Yao Ming has re-analyzed the MRI of 400 patients. She noted that in the presence of extensive white matter lesions, the decreased brain volume is smaller than in the general adult population. This could prove that these white matter lesions reflect, at least in part, the presence of edema (more water) in the brain.

Hypertension is a risk factor for diseases of small arteries that may have a negative impact on small vessels, regardless of Cadasil disease. Cadasil is a disease of the arterial wall resulting in abnormal vasoconstriction. This could suggest that the blood pressure profile of patients could have an impact on the brain and the evolution of the disease.

But the occasional blood pressure measurements made during consultations at CERVCO are not sufficient to assess the impact of blood pressure profile of a person on the evolution of the disease and to detect possible occurrence of drops in blood pressure. To better understand the blood pressure profiles may be useful for the treatment of disease.

Dr. Hervé has submitted a project for the CERVCO team to organize a blood pressure measurement over a full day for all patients followed by the reference center. A prescription will be sent to a cardiologist to get a Holter blood pressure monitor for each patient. Each will be assigned a monitor device (Holter), which will be set for all in the same way to measure blood pressure. This device will be worn for 24 hours, with a cuff. It will take regular measurements, for example every quarter hour during the day and every half hour at night. For this project, the CERVCO team has received advice from hypertension specialists from Georges Pompidou Hospital.

Data such as means, deviations between minimum and maximum values, falling blood pressure, etc.., will be analyzed to establish a link with disease progression. Mr. Abbas Taler, clinical research associate, will be involved in organizing the collection of these measures and to analyze the results.

3 –Questions and Answers

"Can the disease or the consequences of strokes, cause tinnitus?"

Answer: A small lesion of the auditory nerves of vascular origin could cause hearing loss in the course of CADASIL. Some patients experienced sharp reductions hearing. This is probably because small vessels of the

hearing organ are slightly affected. But it is extremely rare that there are experiences of tinnitus for long duration. In cases of hearing loss, do not wait to make appointment with a specialist and consider a hearing aid device if necessary.

"Can the disease or the consequences of strokes, cause urinary problems?"

Answer: A small vessel disease with white matter lesions may be the cause of urinary troubles such as urge incontinence (difficulty holding back, pressing need). But there are different possible causes (urinary tract infections, prostate, effects of births, etc.), all of which require medical examinations.
At an advanced stage of disease, when there are problems with walking, there may be episodes of urinary retention. Medications can help improve them.

"Cadasil France had given CERVCO a grant for a device to measure the walking speed. Has it allowed identifying a trend among monitored patients?"

Answer: Data were collected for several dozen patients. It is necessary to continue these measures and carry on for these individuals over several years in order to draw conclusions. A link should be established with other parameters from the patient's follow-up study and with abnormalities detected on MRI.

"My dentist is about to pull a tooth and wants me to replace Plavix for a certain period before and after surgery. What do you think? "
and "Aspirin is recommended. What is the risk/benefit ratio, especially in bleeding?"

Answer: Professor Bousser recommends aspirin, except for persons who are at risk of bleeding, but otherwise it is advisable for those with a risk of clogged arteries. Aspirin reduces risks of migraines, clogged arteries, myocardial and cerebral infarctions (and their severity), cancerous colon polyps, recurrent cancer, etc.. The recommended dose is 75 mg per day taken with meals. In Cadasil, bleeding risk is low. Increasingly, surgeons and dentists carry on their operations without asking to stop the aspirin.

Plavix is an antiplatelet drug, more expensive, which prevents platelets from sticking to the wall and arteries to clog. But it acts on platelets by a different route. It is recommended for people allergic to Aspirin, or having a stomach ulcer. After myocardial infarction, cardiologists often prescribe a combination of the two most powerful treatments, Aspirin and Plavix, but there is the side effect risk of bleeding.

In Cadasil, the risk of cerebral hemorrhage is very low. But in Cadasil, when an artery gets blocked after a small infarct, it is unclear if a clot or if the artery has a spasm. When in doubt, it is not dangerous to take some aspirin.

Surgeons do not like Plavix, some people are very sensitive and will bleed a lot, it is unpredictable. They are asking to stop this treatment five days before surgery, if there is a risk of bleeding.

Paracetamol is an analgesic i.e. painkiller, it does not prevent arteries from clogging.

"Can the treatment NeuroAiD, derived from Chinese medicine, be effective to recover after a stroke? Can it be prescribed by a neurologist or is it only sold online? It seems expensive?"

Answer: (Mrs. Bousser): a French company based in Singapore markets on the Internet indeed a product of traditional Chinese medicine that would improve both the recovery after a heart attack or a cerebral hemorrhage. There has been no study of effectiveness according to international standards so far comparing the effects of this substance to placebo, but one study (CHIMES) is in progress on 1,000 patients in Asia. The result will be available in a year. It is advisable to wait for these results.

Tolerance seems very good. Test data on experimental animal models of cerebral infarction and in cultured neurons by an advanced French researcher suggest that this substance would be effective. It has phased out some of the fourteen ingredients to keep only some, of vegetable origin; a similar efficiency was observed. He is wondering whether this treatment could also have an impact on degenerative diseases of neurons.

This product marketed on the Internet as a "dietary supplement" is indeed expensive.

Given the point where we are, it is recommended to wait for scientific conclusions. But everyone can make a choice and the effectiveness of this traditional treatment seems at least as convincing as the ads from the pharmaceutical industry for several decades, after very expensive studies.

"In 2008 you said, regarding statins," it is desirable, based on current level of knowledge, to consider statins only in the presence of exceedingly high cholesterol ... ". What is your opinion about them now (in 2012) and why?"

Answer: Prof. Bousser indicates that a study on people with no cholesterol, no history of myocardial infarction and having had a cerebral infarction (not specifically related to Cadasil) showed that statins reduced the risk of infarction.

But statins have been mainly developed for atherosclerosis (condition in which an artery wall thickens as a result of the accumulation of fatty materials such as cholesterol) and Prof. Chabriat confirms his position: He does not recommend statins, except for high cholesterol.

"Is there a possible link between these three diseases: CADASIL, lower limb arteritis and ankylosing spondylitis? According to the information we had, the mutated Notch3 gene is the cause of the fragility of the arteries?"

Answer: The Notch3 gene is present in all vessels of the body. It has a particular function in cerebral vessels, where there is a neuro-vascular coupling. Its mutation has an impact on this neurovascular function and it is not causing problems in clogged vessels in other organs. There is no link with lower limb arteritis or with ankylosing spondylitis.

"Is that possible that CADASIL patients may come from the same "original family"?"

Answer: No, it is not possible. A family who share the same mutation in the Notch3 gene may be different from other families. In France, some families have similar mutations, but overall, the disease appeared in different families with specific mutations in each case.

Some mutations are more common in France and as a result, one may assume that some of these families are linked to a single genetic defect at the origin.

The disease appears more in new families (chance of a birth, parents are not carriers, these are "de novo" cases).

"What is the course of the disease, knowing that each patient's disease presents itself in different ways?"

Answer: The evolution of the disease in a patient is not predictable. The monitoring protocol is aimed to study this evolution. What we observe is that for a long time, the disease (its clinical status) changed little. In general, when the disability (walking disorder, difficulties in concentration and memory) begins to manifest, there is acceleration in the evolution. A stroke is not a sign of worsening in functional terms; the recovery is good in early stage of the disease. It is the accumulation of small infarcts, which explains the severity of the disease.

Very atypical cases have been encountered: some people who carry the mutation show no symptoms at age 70.

"How can the disease be tracked since we were not contacted for an appointment in 2012? (Regular MRI, visits to a neurologist, health check-up including memory and cognitive functions? "

Answer: You should contact the CERVCO secretary at 01.49.95.25.91 and/or secretariat.cervco@lrb.aphp.fr)

"What can be covered? 100% Cadasil support? My husband is currently being covered only for Arteritis: physical therapy is for Cadasil, regular pedicure is at our expense because there is no question of foot care itself due to arteritis?
And therapy to help the patient? Can it be covered? If so how to choose a therapist? "

Answer: Following the General Meeting, Mrs. Morel, part-time social worker at CERVCO, communicated to the President of the Association a document to answer questions. She can be contacted to answer your questions at 01 49 95 65 41.

- 100% reimbursement covers actions and care referenced in the National Protocol for Diagnosis and Treatment (This document was written by experts with the help from the Patient Association). These are recommendations endorsed by the Health Authority, within the rates limits set by Social Security.

- 100% coverage is usually granted only for a few years and it is the patient's responsibility to apply for renewal, through his/her primary care physician. The 100% coverage does not cover the excess fees, health expenses not related to Cadasil, the fixed co-payment of € 1, medical deductibles of € 0.50 and the fixed daily hospital contributions.

- Patients whose condition does not justify 100% coverage are reimbursed at 80 or 60% by Social Security and Private Supplemental Insurance finance the remaining amount, subject to conditions of each insurance company.

- A therapy in a public health facility (hospital or mental/medical center) is supported as part of the coverage where the patient receives (100% or social security & insurance). The same applies if one uses a therapist who is registered in the General Classification of Professional Acts just as a psychiatrist, a General Practitioner. On the other hand, visit sessions with a therapist who is not officially registered in the group will not be eligible for reimbursement.

- The foot care is not covered except for some patients with diabetes. If consultation and foot care were prescribed by a doctor, Social Security may pay about one Euro, which may help trigger the co-payment by an insurance company, according to the insurance policy.

"We advised my husband's brother a screening for CADASIL. His primary physician did not know what it was but sent him to a neurologist in the Paris area (neurologist who did an internship at Lariboisière): He has simply made an examination (touching nose with finger, reflexes, walking ....) and asked some questions and told him he was not suffering from CADASIL;
Is a simple exam like this enough to determine CADASIL? Or should he do more tests at Lariboisière (and that if he wishes)?"

Answer: For the diagnosis of the disease, a simple consultation is not sufficient. A MRI exam showing lesions in certain brain areas quite characteristic of Cadasil can be very suggestive of the disease for a person belonging to a family in which Cadasil has already been diagnosed. But only the genetic test can confirm this.

You can recommend that he contact CERVCO directly, if desired, for a diagnostic procedure, as part of a multidisciplinary consultation.

An initial appointment with a neurologist, a geneticist and a psychologist will help inform and advise him in this approach. After a cooling off period, blood sampling will be conducted. At any time, he may waive his approach. Communication of results is made at a third consultation.

This multi-disciplinary consultation and follow-up to conduct the genetic test was a subject of a CERVCO publication in early 2012 in a leading reference magazine in the United States. It is critical to limit the impact when the diagnosis is announced.

"Is bone marrow donation possible if you are suffering from Cadasil?"

Answer: This question has not been considered yet. It should be evaluated by the medical team before communicating a response.

"What attitude should we adopt with young people who intend to become parents when there is the risk that they are suffering from Cadasil and transmitting the disease?"

Answer: This issue raises many questions, human and ethical. For a couple who is considering giving birth to a child, while the parent, father or mother, has a 50% risk of carrying the mutated Cadasil gene, we must first ask the following questions.

- The couple wants to know if either is inherited with the mutated gene from their parents? And if he/she knows that one is a carrier, how to initiate procedure to avoid transmission? In this case, the couple must apply to a center of Prenatal Diagnosis (PND) (there is one in each university hospital) where they will be provided with assistance to refine their thinking, at a meeting with a geneticist, a gynecologist-obstetrician and a psychologist. When pregnancy is started, a sample will be made around the tenth week on a small piece of placenta, which is the tissue surrounding the fetus. Following the genetic analysis of this sample, prospective parents may be faced with a situation to decide an abortion if the future child is a carrier of the genetic defect. They must have previously considered

this possibility. If the couple is not ready for abortion, what is the point of knowing and also to undergo the risks of abortion relating to the sampling of the placenta?

- On the other hand, if the couple does not wish to know their genetic status and an in vitro fertilization (IVF) will select an embryo which has been verified as not being a carrier (Preimplantation Genetic Diagnosis -PGD method), without telling parents if the embryos were not selected because they had inherited the mutation and in this case one of them would learn that he/she is a carrier.

  This method is not simple; the mother must undergo hormone treatments to stimulate ovulation. However, the stimulus loses its effectiveness at the age of 30 years and has less chance of achieving pregnancy in a mother at 35. The procedure is cumbersome and time-consuming. It often requires several attempts. Psychologically, for parents, they don't have to face a decision for abortion.

  On the Ile-de-France area, a pathway for PGD (Preimplantation Genetic Diagnosis) has been established. Contact Information for specialized centers are published on the CERVCO website or can be requested with the secretary.

The law allows these approaches in the case of a severe disease for which there is no cure. Geneticists take into account another factor: early manifestation of the illness, which is not the case for Cadasil.

And when Prenatal Diagnostic (PND) or Preimplantation Genetic Diagnosis (PGD) are considered, always ask the couple to be prepared to deal with this subject, since the disease occurs only in adulthood, with some manifestations milder and atypical.

The support guidance by psychologists is very important.

For parents of young couples who are asking these questions, it is important to support them in their thoughts, without taking sides or trying to influence them. Access to information on this topic should be made available, while respecting the couple's personal journey.