New approach which can help to predict neurodegenerative diseases

26 July 2010

New investigations, initiated by research workers at CIC bioGUNE and led by Dr. Aitor Hierro, have opened possibilities for making progress in the knowledge and prediction of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS), according to the prestigious journal of *The Proceedings of the National Academy of Sciences* of the United States of America.

This study opens the possibility of research in humans to detect this pathology before the symptoms arise.

The research was undertaken in collaboration with groups from the National Institute of Health (NIH) and the Neuromuscular Center at the Cleveland Clinic, both in the USA, as well as from the National Centre for Scientific Research (CNRS) in France. They have described, for the first time ever, the structure of a protein known as Vps54, one of the four making up the GARP complex of proteins.

Using the wobbler mouse as a model, the protein Vps54 has a mutation which gives rise to a progressive degeneration of the motor neurones and to infertility. Given this characteristic phenotype, the wobbler mouse has been used since its discovery in 1956 by Dr. Falconer as a model for the study of the spontaneous degeneration of motor neurones, including *Amyotrophic Lateral Sclerosis*.

In 2005 Dr. Jockusch's team managed to identify the mutation responsible for the wobbler phenotype. From this moment the team led by Dr. Hierro focused on understanding how this mutation affected the protein and its own activity.

In this way it was discovered that the disease is not only the consequence of a mutation of a concrete protein — a thesis held to date on the basis of previous studies —, but that there exists a domino effect in which reduced levels of the mutated protein have a destabilising effect on the rest of the components of the GARP complex of which they form part. "The illness develops not only because of the mutation but it may also be due to other mutations or defects that generate reduced levels of the GARP complex or instability therein", explained Dr Hierro.

There exists a study undertaken at the University of Michigan by Dr. Brown with 96 patients suffering from sporadic ALS and in which this mutation present in the wobbler mouse was not found and so it can be concluded that this mutation has no great presence amongst humans. "Nevertheless, the great similarity between the GARP complex proteins of the mouse and amongst humans means that such that a motorneurodegenerative effect in humans due to reduced levels of the GARP mooring complex cannot be discarded This opens the possibility for studying these levels in humans where, moreover, it will be possible to predict the illness well before the appearance of symptoms", explained Dr Hierro. "It is highly likely that many patients with some motorneurodegenerative disease do not have the same mutation as the wobbler mouse but there does exist the possibility that, due to some other reason, some patients may have reduced levels of the GARP complex, a situation which gives rise to the illness", stated Dr Hierro.

To carry out this research, various techniques such as x-ray crystallography, together with bioinformatic analysis, have been used, enabling resolving for the first time the structure of the mentioned protein in the region where the mutation is produced. Subsequently a combination of *in vitro* experiments were undertaken using biophysical techniques, as well as *in vivo* ones using various mouse cell lines and tissues with the wobbler phenotype, and it was confirmed that the mutation produces a destabilising effect on the Vps54 protein, an effect that is subsequently transmitted to the rest of the components of the GARP protein complex.
Regulation of cell 'traffic'

The metabolic balance of the cell largely depends on the correct transport of components amongst the various cell organelles, something similar to the great transport networks between cities or ports. The cargo 'vehicles' used for this transport are mostly the vesicles, small lipid spheres that arise from the cell organelle and are transported to another cell organelle. This transport is exquisitely regulated and the defects that may arise could be the cause of the illness.

Many proteins act together for sustaining life, forming what are known as protein complexes (a series of proteins that develop a specific function). The GARP complex is the transport network that recycles receptors of acid hydrolases from the lysosomes (the stomach of the cell) to the Golgi apparatus. Acid hydrolases are enzymes which, in the acidic medium of the lysosomes, are activated and digest other proteins. To avoid the receptors themselves being digested by the acid hydrolases, once the interior of the lysosomes is acidified, these recycle to the Golgi by means of transport vesicles. In this way, the receptors are recycled and reused to transport new acid hydrolases to the lysosomes. In concrete, the GARP complex is formed by four proteins and is responsible for physically tying up the vesicles that are transported to the destination organelle, in this case the Golgi apparatus, something similar to a vessel being moored at a port. Subsequently the vesicles fuse to discharge their transported content.

The study concluded that the wobbler phenotype is the consequence of a drastic reduction in the levels of the GARP complex, one that is required to tie up the transport vesicles to the Golgi apparatus. This drop in the levels of the GARP complex thus breaks with the normal functioning of the recycling route of the acid hydrolase receptors to the Golgi apparatus.

Provided by Elhuyar Fundazioa