19º International symposium on ALS/MND
Birmingham – United Kingdom
3rd – 5th November 2008
The International Symposium on Amyotrophic Lateral Sclerosis (ALS) was held in Birmingham, UK, on November 3rd-5th 2008.

This year 860 participants worldwide were present in this symposium and at least 300 abstract were submitted. Moreover, non-profit institutions were present, including “MNDA” (Motor neurone disease association); “Hope”, “Patients like me”, “ABrELA” (Brazilian Amyotrophic Lateral Sclerosis Association) and also Institute Paulo Gontijo, which presented the next Paulo Gontijo Award to be given in 2009.

All abstracts can be downloaded from the MNDA website:

Introduction

The Amyotrophic lateral sclerosis, also known as motor neurone disease and Lou Gehrig disease, is a group of neurodegenerative diseases which affect mainly the motor neurones. The motor neurones degenerate with the disease progression causing muscle weakness and speech, swallowing and breathing problems.

There are similar diseases with the same symptoms as ALS and the correct diagnosis occurs excluding other diseases after a series of tests.

We can classify ALS as familial and sporadic forms. Familial ALS is caused by mutations in different genes including SOD1 (superoxide dismutase 1), Alsin, Senataxin, VAP-B, Angiogenin and TARDBP. These mutations are distributed throughout the family with more individuals affected with the same disease. On the other hand, sporadic forms are the most common form and only the index case is affected, i.e., no more individuals in the family are affected. Mutations in genes described above can cause sporadic ALS as well as unknown factors.

Cellular biology and Pathology session

Angiogenin

Recently Matthew Greenway and colleagues found a new gene involved in ALS (familial and sporadic forms).

Angiogenin was first identified as a molecule involved in blood veins formation (angiogenesis). Its function is similar to VEGF (vascular endothelial growth factor), which seems to be a protective factor to ALS.

Vasanta Subramanian showed angiogenin is expressed in early stages of embryonic development, in special motor neurones. Moreover, this protein seems to be important for neurites migration.

Angiogenin organizes endothelial cells into tube-like structures, which helps cellular migration during tissue vascularisation. Also, this protein seems to be involved in other cells proliferation.

Its function is not completely known and we still do not know how it causes ALS. Future studies will be important to understand the involvement of this protein in ALS pathology.
In another study including angiogenin, Michael Van Es from Holland studied 39 families with ALS and 275 normal controls.

In one of these families, he found a mutation (K17I) in three patients of this family, but it was not present in the controls. All patients of this family had a fast progression.

One individual of this family is 72 years old at the moment and was diagnosed with Parkinson’s disease five years prior diagnosis of ALS. Recently he showed inappropriate sexual behaviour alteration and a new diagnose was determined as Fronto-temporal dementia (FTD).

It is not known if this patient was screened for mutations in TARDBP gene or if there is any association between angiogenin and FTD.

**Retinoids and ALS**

Christi Kolarcik and colleagues studied the effect of retinoids in ALS. The main aim of their study was identify and explore the functional effects of retinoids and ALS and also verify its effects in motor neurones.

They studied post-mortem material from ALS patients and they verified the expression and distribution of retinoids in cells.

Retinoic acid is an important factor for neural development, specificity and neuronal plasticity to repair damaged neurones.

The authors showed difference in three proteins: cellular retinol binding protein (CRBP) and cellular retinoic acid binding proteins (CRABP) I and II.

CRBP expression was increased in motor neurones of spinal cord from ALS patients while CRABP-I was decreased. In normal situation CRABP-II is located in the cytoplasm however in patients with ALS, this protein is relocated into the nucleus of the cells.

In addition, the authors showed expression and distribution of retinoids and its nuclear receptors. They tested receptors for retinoic acid alpha, beta and gamma in the cells. The Alpha and gamma receptors showed nuclear staining; however beta receptors are dramatically increased in ALS sporadic patients. It was observed in 66% of sporadic cases and only in 11% of familial cases.

Also they showed the exposure of retinoic acid in the neurones increases the number of neurites.

They conclude that the retinoic acid pathway is involved in ALS and is a potential target for future treatments.

**Laser capture and microdissection and expression arrays in transgenic animals**

John Ravits from Washington University showed that is possible to enrich motor neurones samples using laser capture microdissection. Samples from the spinal cord of transgenic animals with SOD1 mutation were processed in different time points (20, 60 and 90 days).

The main goal of his study is to verify which genes are expressed before and after these animals develop ALS.

A list of different genes was provided including apoptosis, structural and cellular signalling genes.

There is a difference in the gene expression in different time points as expected and the authors are analyzing it at the moment. They believe that
this study will help to understand the connexions between genes and it will be a big challenge to validate the data.

**Exercises and ALS**

Recently Italian football players were diagnosed with ALS. It is not the first time that sports are associated with ALS. The first case was reported in USA when Lou Gehrig, a famous baseball player developed ALS.

Laura Ferraiuolo from Sheffield University showed that animals at 12 weeks of age were trained every day to run during 3 weeks. The first group ran in average 13 km for at least 5 hours per day while the second group ran only 1 km per day.

After three weeks of training, spinal cord of these animals were isolated using laser capture microdissection and RNA was obtained. The RNA analysis showed 444 genes differently expressed, 203 of them were overexpressed and 241 were downregulated. Neurotrophic factors and its receptors, ion channel regulators and cytoskeleton genes were among them.

The authors also studied muscle from these animals and verified that 370 genes were altered, including metabolism and angiogenic genes, and VEGF-2. Also they report a gender difference in which females tend to run more than males.

It is not known why there is this difference between males and females and also sports and ALS.

The authors believe that individuals with ALS predisposition associated to regulation of neurotrophic factors and sports could trigger ALS in these patients.

**Axonal transport: VAP-B and Spastin**

**VAP-B**

Kurt de Vos from King’s College London showed that mutations in VAP-B (vesicle associated membrane protein-associated protein B) gene alter axonal transport of this protein.

VAP-B was first identified in a large Brazilian family in 2004. This protein is located in the membrane of endoplasmic reticulum and mitochondria. The mutation leads to formation of intracellular aggregates and a mislocalization of this protein.

There are two axonal transport pathways: anterograde transport in which proteins from cellular body is transported to dendrites and retrograde via in which proteins from dendrites are transported back to cellular body.

The transport of proteins and organelles has involvement of other proteins such as kinesin and dynein.

Using mouse cortical neurones De Vos showed that mutation in VAP-B causes a decrease in the number of mitochondria transported. In normal situation 30% of mitochondria are transported, but with the mutation this number decreases to 10-15%. The majority of the mitochondria with mutation remains in the cellular body and few are observed in the axon and neurites. The retrograde transport is normal.

In addition he showed that the transport speed is constant indicating that the motor proteins are normal.
The author concludes that the mutation inhibits the anterograde transport only.

**Spastin**

Paul Kasher using the same technique as described by De Vos, showed that spastin alters anterograde transport.

Mutations in the *spastin* gene cause spastic paraplegia (SPG4). Spastin interacts with microtubule, responsible for the cytoskeleton formation. Mutations in this gene cause axonal swellings, structures formed by cytoskeleton swelling and sequestration of organelles and vesicles in the axon of neurones. The axonal swelling blocks the transport of proteins throughout the axon leading to motor neuron death.
Kasher studied the mutation K388R in spastin gene and it causes the formation of a non functional protein. The author showed that this mutation causes an alteration in the anterograde transport.

**Development of new drugs for ALS**
Thierry Bordet showed that TRO19622 could potentially be important for treatment of ALS.
This drug protects motor neurones from injuries caused by deprivation of neurotrophic factors in cell culture. Besides it accelerates in 54% the recovery of auxotomized nerves in animals.
TRO19622 delays the symptoms in transgenic animals with SOD1 mutation and alters the microtubules assembly, extending it into 20%.
This drug was tested in animals with no collateral adverse and will be tested in ALS patient in near future.
More information about this drug is available in the website below:

Another target for future treatments involves the NRF2/ARE pathway.
No details were provided in this symposium, however the authors believe in near future they will develop a new drug for ALS.

**TDP-43**
In 2006 a new protein was found altered in spinal cord of ALS and FTD patients. There is no clear association between these two diseases however mutations in TARDBP gene can cause both.
This gene produces a protein called TDP-43 and is normally localized in the nucleus of the cells. The protein is involved in RNA regulation, splicing and interaction with other RNAs and proteins.
Jemeen Sreedharam from King’s College London showed that mutations in TARDBP gene cause ALS in patients from UK and New Zealand.
The M337V mutation was found in patients from one family and the Q331K mutation was found in a sporadic case.
Vineeta Tripathi from the same group introduced these mutations in chick embryos and verified that the mutation causes delay in the development of the embryos and premature death.
By Dr. Vineeta Tripathi

Figure 2: Effect of overexpression of TDP-43 protein in chick embryos. Embryos electroporated with normal gene do not show alterations in development and morphology (A). However 50% of embryos expressing the mutation M337V B) and Q331K (C) showed a delay in the embryos’ development (See tails and limbs).

Animal models

VAP-B in fruit flies
Giuseppa Pennetta from Edinburgh University showed that mutation in VAP-B homologue gene in fruit flies causes locomotion impairment. The mutation causes aggregates in the flies neurones and alteration in the microtubule assemble.

SOD1 and dogs
Joan Coates showed dogs with neurodegenerative disease. These animals have spontaneous mutations in SOD1 gene and may be an alternative animal model for ALS.

Genetics and ALS
In genetics session, several groups showed results from wide genome association studies in American and European population. The authors showed alterations in KIFAP3, DPP6 e FBX08 genes and they conclude that is necessary to replicate these studies and the need of a larger number of patients to validate their results.

Stem cells therapy
In the last session of this symposium, Clive Svendsen presented briefly his research using the iPS cells (induced pluripotent stem cells). The iPS cells were created by Shinya Yamanaka in 2006 and this technique consists of switching on four genes in skin cells (fibroblasts). These genes are introduced in the fibroblasts and after few months these cells become stem cells-like. It is possible to differentiate stem cells into motor neurones, however only 10% of stem cells become motor neurones. This is problematic if one wants to replace dead motor neurones. Alternatively is possible to introduce
growth factors such as GDNF (glial cell line-derived neurotrophic factor) in dying motor neurones and it will survive longer.

He believes that a therapy for ALS is possible in near future using a combination of growth factors and replacement of dead cells.

At the moment there is no such treatment available.

Vaccine for ALS

Jean-Pierre Julien presented his preliminary data about vaccines for ALS in the last presentation in this symposium.

His group is producing different types of antibodies specific for each mutation in gene SOD1 and he expects to start testing these antibodies in transgenic animals soon.

Dr Agnes L. Nishimura was invited to participate in the 19º International Symposium on ALS/MND by Institute Paulo Gontijo.