

## **Innovative approaches to improve adult brain's self-repair: implications for neurodegenerative diseases**

M.P. Abbracchio

Department of Pharmacological Sciences, University of Milan, Italy

It is now widely accepted that the generation of new neurons (neurogenesis) and new glial cells (gliogenesis) is not restricted to the period of central nervous system development and maturation, but also occurs during adulthood. Multi-potent quiescent stem-like cells that, under appropriate conditions, can differentiate to the three main neural cell types (ie, neurons, astrocytes and oligodendroglia) are present not only in adult brain's neurogenic areas (the subventricular zone of lateral ventricles and hippocampal dentate gyrus) but throughout the brain's parenchyma. Upon appropriate stimuli, these cells can assume stem-like properties (Buffo A et al, Proc Natl Acad Sci USA, 102(50):18183-8, 2005; Proc Natl Acad Sci USA, 105(9):3581-6, 2008). Thus, the full knowledge of the intrinsic and extrinsic factors that regulate the functional activity of these cells can lead to the development of new therapies to generate new neurons and new glia able to replace dead cells in acute and chronic neurodegenerative diseases.

Our laboratory has been involved for several years in studying new means to foster the ability of the brain to repair itself.

We have recently reported the orphanization of a new receptor, GPR17, that specifically responds to "danger signals" (Ciana P et al, EMBO J, 25(19):4615-27, 2006), ie, endogenous molecules, such as extracellular nucleotides and cysteinyl-leucotrienes, which are released in great amounts at the site of damage after various kind of injury. These signalling molecules do not only activate immediate local defense mechanisms, but also participate to the subsequent lesion remodelling and repair. Specifically, by utilizing different experimental models of brain injury, we have shown that immature GPR17-expressing cells accumulate in the peri-injured area close to ischemic, traumatic or demyelinating lesions, as well as close to neurodegenerative plaques in Alzheimers' brains. We have also shown that stimulation of GPR17 via its specific endogenous ligands activates quiescent progenitor cells that start proliferating and differentiating in order to generate new oligodendroglia, which can, in turn, ensheath neuronal axons to repair dysfunctional myelin and restore nerve conduction. To confirm a crucial role for GPR17 in myelination, addition of GPR17 ligands to oligodendrocyte precursor cells in culture promotes their differentiation to mature myelinating cells (Lecca D et al, PLoS ONE, 3(10):e3579, 2008).

Our results have been recently confirmed by an independent study suggesting that GPR17 may act as an intrinsic regulator of myelination in oligodendrocyte precursor cells (Chen et al., Nat Neurosci, October 18, doi:10.1038/nn.2410). We are currently developing new GPR17-selective pharmacological agents that could be used to stimulate the terminal differentiation of brain neural precursor cells, thus implementing the brain's self-repair abilities in acute (trauma, ischemia) and chronic disorders (Alzheimer's disease and multiple sclerosis).

## **Altered stress oxidative profile in cortex of mice fed by a BCAA diet**

A. Adduci, P. Piscopo, A. Crestini, A. Ferrante, P Popoli, N. Vanacore and A. Confaloni

Department of Cell Biology and Neurosciences; CNESPS, Istituto Superiore di Sanità; Department of Therapeutic Research and Medicine Evaluation, Istituto Superiore di Sanità, Italy

**Introduction** Branched-chain amino acids (BCAAs) are widely used among athletes as dietary integrators to improve physical performances. Several evidences show that defects of the BCAAs metabolism can cause some neurological effects. Recent epidemiological studies have also shown a much higher risk for Amyotrophic Lateral Sclerosis among Italian professional soccer players, in comparison to the rest of the population (Belli and Vanacore, 2005). The aim of the study was to explore the effects of a BCAA diet on the expression profile of genes involved in oxidative stress.

**Materials and Methods** Eight mice received a Basic Standard Diet while eight mice received an experimental diet enriched in 2.5% Val:Leu:Ileu/1:2:1. Brains were removed and dissected in cortices. The molecular analysis was performed by the RT<sup>2</sup> profiler PCR array and Western Blot analysis to evaluate the protein levels.

**Results** We observed that out of 84 genes analyzed, just twelve of them were differentially modulated. In particular, an enriched BCAA diet down-regulates the expression of six antioxidant genes, while up-regulates the expression of six oxygen transporters. Moreover, SOD1, a Familial ALS-related gene, showed a down regulation at transcriptional and translational levels.

**Conclusions** Our results suggest that a BCAA diet is able to modulate gene expression in mammalian brain, altering some oxidative stress pathways.

## **Telomere length modulation in astroglial brain tumors**

M. Aguenouz., D. La Torre, A. Conti, D.Pontoriero, C. Tomasello, S. Romeo, A. Ciranni, G. Vita  
Department of Neurosciences, Psychiatry and Anaesthesiology, University of Messina, Italy

**Introduction** The phenomenon of telomere alteration during tumorigenesis process and progression of solid tumors is well known and established at the molecular level. Several pathways have been identified that regulate telomere length in human tumors. However, the phenomenon of bidirectional telomere dysfunction, including attrition or elongation, is subjected to the same overarching characteristic for almost all solid tumors, i.e. heterogeneity. The present study was designed to clarify the pathways that modulate telomere maintenance in astroglial brain tumors.

**Materials and Methods** A cohort of 38 flash-frozen surgical specimens, obtained in adult patients who underwent craniotomy for microsurgical tumor resection, histologically verified as grade 2-4 astrocytomas, was used for the study. The Authors studied terminal restriction fragment (TRF) length, and the expression levels of a panel of genes controlling the length and structure of telomeres. The correlation among the levels of genes expression, telomere length, and histological grading, were studied as well.

**Results** Up-regulation of TRF1 and shorter telomere resulted in the low grade gliomas (LLGs), While, down-regulation of TRF1 and up-regulation of both telomerase and PARP1, resulting mainly in High grade (HGGs). Moreover, a statistically inverse correlation between TRF1 binding proteins and telomere length was found.

**Conclusions** These findings support the hypothesis that in human astroglial brain tumors typical biomolecular features dealing with biological behavior of malignancy may exist.

## Activated microglia as targets for restorative approaches

M.A. Ajmone Cat, P. Cilli, S. Biagioni, E. Cacci, L.Minghetti

Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità;  
Department of Cell and Developmental Biology, “La Sapienza” University, Rome,  
Italy

**Introduction** The complex process of microglial cell activation encompasses several functional activation states associated with neurotoxic/anti-neurogenic or neurotrophic/pro-neurogenic properties, depending on many environmental factors including nature and duration of the activating stimuli. A main goal of regenerative neuroscience is understanding the mechanisms behind the neuroprotective effects of microglial activation in order to enhance endogenous self-repair mechanisms and limit neurodegeneration. We previously demonstrated that repeated and prolonged (72 hr) in vitro exposure to lipopolysaccharide (LPS) endows microglia with a potentially neuroprotective phenotype, here referred as to “chronic”.

**Materials and Methods** Rat microglial cultures and hippocampal organotypic cultures were exposed to acute (24h) and chronic (72h, with 3 subsequent 24h-challenges) LPS stimulation. Cytokine production was assayed by real time PCR and specific ELISA. Neurogenic activity was tested on embryonic precursor cell cultures (NPC) and on hippocampal slices.

**Results** The synthesis of proinflammatory cytokine interleukin (IL)-1alpha, IL-1beta, IL-6, and tumor necrosis factor (TNF)-alpha was strongly reduced after chronic stimulation of microglia, as compared with acute stimulation. At variance, IL-10 and prostaglandin E2 levels remained elevated or were further increased after chronic LPS exposure. Acutely activated microglia, or their conditioned medium, reduced NPC survival, prevented neuronal differentiation and strongly increased glial differentiation. Conversely, chronically activated microglia were permissive to neuronal differentiation and cell survival.

**Conclusions** Our findings suggest that, in a chronically altered environment, persistently activated microglia can display protective functions that favor rather than hinder brain repair processes.

## **Characterization of a novel PSEN2 transgenic murine model for Alzheimer's disease**

A. Albanesi, A. Crestini, D. Albani, P. Piscopo, S. Batelli, M. Sbriccoli, G Forloni and A. Confaloni

Department of Cellular Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy; Department of Neuroscience, M. Negri Institute for Pharmacological Research, Milan, Italy

**Introduction** Transgenic mice overexpressing mutant familial Alzheimer's disease genes have contributed to an understanding of dementia pathology. Presenilins (PSEN1 and PSEN2) influence multiple molecular pathways and are known for their role in the  $\gamma$ -secretase cleavage.

**Materials and Methods** We have generated PSEN2 transgenic line under transcriptional control of the PDGF promoter expressing the human Ala85Val PSEN2 variant found for the first time by our group, in a Sardinian family (Piscopo et al. 2008). We set out to characterize the new PSEN2 transgenic mouse model in order to better understand the influence of the A85V mutation on murine phenotype expression. With this aim we have studied the transcriptional levels of PSEN2 in several systemic and brain districts of mutant and wild type mice by Real Time. Furthermore we analysed presenilins level by western blot and immunohistochemistry in several cerebral districts.

**Results** We observed that mutant hPSEN2 is expressed in all systemic and brain districts analysed according to the molecular data. In particular, we observed a greater expression of our transcripts in the hippocampus and olfactory bulbs.

**Conclusions** These preliminary data support the use of these mice to obtain insights into the dynamics of PSEN2 and successively its effects on A $\beta$  levels in the brain.

## **Two-phase screening to discover genetic polymorphisms in the sirtuins' genes affecting Alzheimer's disease risk**

D. Albani, L. Polito, A. Signorini, D. Galimberti, E. Scarpini, G. Forloni  
Mario Negri Institute; Fondazione Ospedale Maggiore Policlinico, University of Milan, Milan, Italy; Golgi-Cenci Research Center, Abbiategrasso, Milan, Italy

**Introduction** Sirtuins are conserved enzymes with deacetylase activity. Mammal sirtuins are seven (SIRT1 to SIRT7) and are localized in different subcellular compartments: the nucleus, the cytosol and the mitochondria. Sirtuins are involved in pivotal physiological processes as aging, apoptosis, stress response and several data suggest the involvement of SIRT1 and SIRT2 in Alzheimer's disease (AD). We have decided to search for frequent single nucleotide polymorphisms (SNPs) in sirtuins' genes that might be associated to sporadic AD risk modulation in the Italian population.

**Materials and Methods** In a first step, we selected a group of 48 AD and 48 matched controls to be screened by dHPLC (*Denaturing High Performance Liquid Chromatography*) for the presence of SNPs. Aberrant chromatograms have been sequenced to identify the mutation. In a second step, differentially represented SNPs from phase I and a panel of other SNPs covering SIRT1 to SIRT7 coding region taken from the HapMap project (total 35) has been analyzed by Sequenom technology in a group of 190 AD and 380 controls to quantitatively assess their influence on AD risk.

**Results** In AD patients we have found 17 intronic SNPs distributed in the seven sirtuin genes. Moreover, we have also identified some coding polymorphisms, most of them rare (for instance, Glu→Lys at codon 535 of SIRT1, Val→Ile at codon 207 and Pro→His at codon 262 of SIRT3. Phase II analysis are ongoing.

**Conclusions** We have verified that sirtuins show a genetic variability between AD e matched controls in the Italian population and might modulate AD risk.

## **Gene expression study of a novel progranulin splicing mutation in an autosomal dominant early-onset case of frontotemporal dementia**

M. Anfossi, L. Bernardi, D. Bellizzi, M. Gallo, S. Geracitano, R. Colao, G. Puccio, F. Frangipane, S.A.M. Curcio, M. Mirabell, C. Tomaino, F. Vasso, A. Clodomiro, R. Di Lorenzo, L. Benussi, G. Binetti, N. Smirne, G. Passarino, R. M., A.C. Bruni.  
Regional Neurogenetic Center, ASP-CZ Italy; University of Calabria, Rende, Italy; IRCCS Brescia, Italy

**Introduction** Most of the pathogenic mutations in the Progranulin (PGRN) gene associating Frontotemporal dementia (FTD) are nonsense, frameshift or splice-site mutations and result in a premature stop codon and degradation of the mutant RNA by nonsense-mediated decay. We report the molecular characterization of a novel PGRN splicing mutation in a patient with autosomal dominant early-onset familial FTD.

**Materials and Methods** Clinical and neuroradiological assessment were performed; patient's DNA, mRNA and progranulin protein plasma levels were investigated. Pedigree was reconstructed.

**Results** At 55 years the patient manifested severe disturbed behaviour and successively cognitive deterioration. Diagnosis of FTD was done and family history revealed other six affected subjects over three generations.

The patient carried one novel heterozygous splice-site (IVS7g.1871A>T) mutation. Gene expression study of the mutation revealed that it causes premature termination of the coding sequence and degradation of the mutant RNA with a strong reduction of plasma progranulin levels.

**Conclusions** Pathogenic mechanism of the splice-site mutations is confirmed together with the reduction of plasma progranulin protein levels.

## **Role of autophagy and atrophy in different GSDII phenotypes: how a survival response becomes a pathogenetic mechanism**

C. Angelini, A.C. Nascimbeni, M. Fanin, E. Masiero, E. Tasca, M. Sandri  
University of Padova, Department of Neurosciences; Venetian Institute of Molecular Medicine; Dulbecco Telethon Institute, Padova, Italy

**Introduction** Glycogen storage disease type II (GSDII) is an autosomal recessive disorder caused by acid alpha-glucosidase (GAA) deficiency, leading to lysosomal glycogen accumulation, primarily in cardiac and skeletal muscles. Complete GAA deficiency causes a rapidly progressive disease in infants resulting in hypertrophic cardiomyopathy and respiratory failure (Pompe disease). In less severe late-onset forms, cardiac muscle is usually spared and slowly progressive myopathy and diaphragmatic weakness are the main symptoms.

**Materials and Methods** We studied muscle autophagy and atrophy markers in 3 infantile-onset and 15 late-onset GSDII patients.

**Results** Our results indicate that the infantile-onset subjects constitute a homogeneous group, not only for what concerns clinical severity but also with reference to autophagic impairment and atrophic rate. All infantile patients show induction of autophagy and ubiquitin proteasome systems. Vacuolated/engulfed fibers display autophagy-related atrophy, suggesting a key role of impaired autophagy and subsequent autophagosomes accumulation in myofibrillar disorganization and alteration of endocytic trafficking.

Conversely, late-onset patients appear to have heterogeneous features, although the majority of them show induction of autophagy and atrophy as well. Interestingly the presence of P62 positive aggregates correlate pretty well with myofiber atrophy. In general the degradation systems appears to be still functioning in these patients and seems to contribute positively to counteract disease progression.

**Conclusions** The present data underline the role of unproductive autophagy and accumulation of aggregate-prone ubiquitinated proteins in the pathogenesis of GSDII, especially in more severely affected patients.

## **SOX2 and cell stemness in gliomas and their cell lines**

L. Annovazzi, V. Caldera, M. Mellai, E. Andreoli, D. Schiffer  
CNBO, Vercelli - Policlinico di Monza Foundation/University of Turin, Italy

**Introduction** SOX2 is a single-exon, intronless gene, located on 3q26.3 – q27 chromosome. It belongs to the SOX family of transcription factors. During embryonic development it is highly expressed in the CNS, downregulated when neural cells exit the cell cycle and differentiate, and it inhibits neurogenesis. It is present in neural stem cells and disappears with their differentiation.

**Materials and Methods** SOX2 has been studied in formalin fixed and paraffin embedded samples of normal brains and 121 operated gliomas (80 glioblastomas, 10 astrocytomas, 10 oligodendrogliomas, 5 medulloblastomas, 4 ependymomas, 12 meningiomas) by immunohistochemistry, immunofluorescence, Western blotting and Real Time PCR. The same procedures were applied to 12 glioblastoma cell lines. The goal was that of defining the role of SOX2 in gliogenesis, in tumor stem cells and in their proliferation.

**Results** By immunohistochemistry, in normal brains SOX2 is not expressed in neurons, with some exceptions, and in adult glia cells. In gliomas it is poorly expressed in low grade tumors and highly expressed in glioblastomas and anaplastic oligodendrogliomas. There is a good correlation between immunohistochemistry and Western blotting. Gene amplification (>3 copy numbers) occurred in 12% glioblastomas and in anaplastic oligodendrogliomas. By immunofluorescence, neurospheres (4 cases) are intensely positive in nuclei and show amplification, whereas in adherent cells SOX2 is cytoplasmic with a lower band by electrophoresis. Almost all tumors from which neurospheres developed show amplification.

**Conclusions** SOX2 is a marker of tumor stem cells, helps to identify them *in vivo* and regulates cell proliferation in the embryonal stage and in tumors.

## **Immunohistochemical localization of Receptor for advanced glycation end products (RAGE) in the R6/2 mouse model of Huntington's disease**

S. Anzilotti, L.Perrone, C.Giampà, D. Laurenti, G. Bernardi, M.A.B. Melone and F. R. Fusco

Neuroanatomy Laboratory, Santa Lucia Foundation IRCCS and Department of Neuroscience, Tor Vergata University Rome; Laboratoire de Neurobiologie des Interactions Cellulaires et Neurophysiopathologie, Marseille; Department of Neurological Sciences, II University of Naples, Italy

**Introduction** The receptor for advanced glycation end-products (RAGE) is a multi-ligand receptor that belongs to the immunoglobulin superfamily of cell surface receptors, whose ligands are known to be upregulated in neuropathological conditions. RAGE upregulation has been described in neurodegenerative diseases, such as Alzheimer's disease, Creutzfeldt–Jakob disease and Huntington's disease (HD) (Ma et al, 2004).

**Materials and Methods** To analyze in detail the implication of RAGE in HD, we studied the immunohistochemical distribution of RAGE in the striatum of the R6/2 mouse model of HD, with particular attention to the neuronal subpopulations and their relative vulnerability to HD neurodegeneration.

**Results** We show that RAGE immunoreactivity is evenly distributed to the cytoplasm of neurons in the wild type mouse, while it is spot-like in the R6/2 mouse. Moreover, RAGE is expressed in the striatum with an uneven distribution that reminds of the striosome pattern, but does not overlap with the calbindin-labeled patch-matrix compartmentalization. RAGE is distributed in 90% of spiny projection neurons, both in the normal mouse and in the R6/2. RAGE co-localizes with all of the striatal interneuron subsets both in the wild-type and in the R6/2 mouse. However, the intensity of RAGE immunoreactivity is significantly higher in the spiny neurons and in the PARV and CALR neurons of R6/2 mouse, whereas it is comparable between R6/2 and wild-type in the cholinergic and somatostatinergic interneurons.

**Conclusions** These data support the concept that RAGE is upregulated in the neurodegenerative process of HD, and suggests that its activation is related to the individual vulnerability of the striatal neuronal subtype.

## **Interactive effects of 17 $\beta$ -estradiol and reelin on Alzheimer's related genes expression**

S. Barbati , P. Piscopo, E. Romano, R. Di Fava, G. Laviola and A. Confaloni;  
Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità,  
Rome, Italy

**Introduction** Reelin (RELN) is a protein of extracellular matrix involved in the regulation of neuronal migration, cerebral development, synaptic plasticity and LTP. It has been suggested that RELN plays a role in some neuropsychiatric and neurological disorders like autism and Alzheimer's disease (AD). Notably, variations in reelin gene seem to be associated with AD onset, and an *APP* increase was detected in children with severely autistic behaviour and aggressivity. We have investigated the expression of some genes involved in AD, as *APP*, *PSEN1*, *PSEN2* and *BACE1* in heterozygous (*hz*) reeler and *wild type* (*wt*) brain cortex. Moreover, as a role for estrogens has been hypothesized, in both pathologies, we also analyzed the effect of 17 $\beta$ -estradiol treatment on the transcriptional modulation of genes linked to the AD pathology in the reeler model.

**Materials and Methods** We used *wt* and *hz* reeler mutant mice producing a reduced reelin expression. Female mice received 17 $\beta$ -estradiol intra-*cisterna magna* on pnd 5 and were analyzed on pnd 7,14, 21 or 150.

**Results** On pnd 150, a significant down-regulation of *APP* was found only in *hz* mice treated with the hormone; *PSEN1* and *BACE1* were not modulated, while *PSEN2* was down-regulated by 17 $\beta$ -estradiol in both *hz* and *wt* mice.

**Conclusion** Our results suggest that early central 17 $\beta$ -estradiol treatment is able to modulate *APP* and *PSEN2* expression in adulthood, while only *APP* gene is linked to a reduced reelin expression in *hz* mice.

## **Prion protein insertion in a family affected by frontotemporal dementia associated to the psen1 V412I mutation**

L. Bernardi, M. Anfossi, M. Gallo, S. Geracitano, R. Colao, G. Puccio, F. Frangipane, S.A.M. Curcio, M. Mirabelli, F. Vasso, A. Clodomiro, R. Di Lorenzo, N. Smirne, R. Maletta, A.C. Bruni.

Regional Neurogenetic Center, ASP-CZ, Italy

**Introduction** Prion protein (PRNP) gene repeat expansion with five to nine extra octarepeats causes early-onset, slowly progressive atypical prion diseases with an autosomal dominant pattern of inheritance and a remarkable range of clinical features. Recently, PRNP were found associated with clinical pictures resembling FTD.

**Materials and Methods** *FUS* family was previously reported as affected by early-onset autosomal dominant FTD segregating the the PSEN1 Val412Ile mutation.

**Results** Sequencing of PRNP gene identified a seven extra-repeat insertional mutation in two of the three affected PSEN1 mutated subjects. Analysis of PSEN1 and PRNP genes in available first and second degree unaffected relatives (age 71, 49, 66, 37 years) revealed two other PSEN1 and two other PRNP mutation carriers.

**Conclusions** PRNP and PSEN1 mutations in this family present with a phenotype resembling FTD. Presence of both mutations at the same time seems associated to a very early onset within the third decade of life, whereas the presence of either PSEN1 or PRNP mutation seems confer a variable or incomplete penetrance, given that the elderly unaffected subjects carried only one of the two mutations. What type of neuropathology underlines this disease remains however to be established.

## **Clinical and histopathological findings in tubular aggregate myopathy**

C. Borsato, L. Bello, M. Cao, V. Romeo, P. Nicolao, V. Codemo, M. Fanin, E. Tasca, R. Stramare, C. Angelini, E. Pegoraro.

Department of Neurosciences, University of Padova, Padova, Italy; Department of Medical Sciences and Therapy.

**Introduction** Tubular aggregate (TA) are inclusions within muscle fibers and are observed in various neuromuscular conditions. TA myopathy may be inherited in an autosomal dominant (AD) or recessive fashion. Clinical features are very heterogeneous ranging from myalgias to progressive proximal muscle weakness.

Our aim was to provide clinical and histopathological characterization of sporadic and familial cases.

**Materials and Methods** 33 sporadic cases and 8 familial cases belonging to 2 families were studied.

**Results** TA were observed both in type I and type II muscle fibers in the sporadic cases. Clinical phenotype included periodic paralysis, myotonic dystrophy, LGMD-myasthenia, malignant hyperthermia, and unspecified myopathy. The inheritance pattern was AD in the two families studied. One family (1) presented with severe myopathy starting early in childhood. Muscle MRI abnormalities in T1 were observed in clinically involved muscles. STIR sequences showed peculiar hypointense areas within muscles. Muscle weakness involved proximal lower and upper limbs and was slowly progressive. The second family (2) presented with myalgia and mild muscle weakness. TA were predominantly in type II fibers in family 1 and in type I in family 2. EM showed subsarcolemmal clusters of single- and double-membrane tubules, hexagonally arranged.

**Conclusions** TA myopathy results in a wide spectrum of clinical phenotypes ranging from myalgia to muscular dystrophy of various severity. TA were observed both in type I and type II fibers. Muscle MRI shows peculiar findings in TA myopathy.

## **Neural stem cells release soluble factors that modulate inflammatory cytokines expression in microglia**

E. Bresciani, S. Caporali, S. Frigerio, L. Tamiazzo, L. Rizzi, A. Torsello and V. Locatelli

Department of Experimental Medicine, University Milano-Bicocca; IRCCS Foundation Carlo Besta, Milan, Italy

**Introduction** Inhibition of microglia-mediated neuroinflammation is an important therapeutic target in order to avoid cognitive and motor impairment in brain ischemia. Reportedly, Neural Stem Cells (NSCs) brain grafts have neuroprotective effects. It has been proposed that these are not caused only by NSCs proliferation and generation of new neurons, but also by a modulation of the lesion environment. Our primary aim was to ascertain whether NSCs were capable of modifying microglial activation *in vitro*.

**Materials and Methods** We used ATP as inflammatory stimuli, since it is massively released from damaged neurons and is responsible of activation of microglia during ischemia.

**Results** We demonstrated that N9 microglia cells incubated with conditioned media (CM) from NSCs culture blunted the response to ATP in term of intracellular calcium release. Moreover, CM-preincubation significantly inhibited the expression of TNF-alfa, COX-2 and IL-10 that are up-regulated after ATP stimulation and increase cell survival. Reportedly, microglial cells can induce the transmigration of NSCs in the lesion site, by the release of soluble factors. The aim of our research is 1) reproduce the transmigration process between N9 and NSCs *in vitro* and 2) identify the chemokines most involved in this process and understand their role by experiments of m-RNA interference.

**Conclusions** Our data demonstrate that NSCs release soluble factors that have an antiinflammatory action blunting the N9 response to ATP stimulation; finally, the RNA-interference technique applicated to MCP-1 could let us to understand whether in the activated N9 cells this chemokine may have a role in the transmigration process of NSCs.

## **Glial cells: critical players in adult neurogenesis and in brain repair”**

A. Buffo

Department of Neuroscience, Neuroscience Institute of Turin, University of Turin

One of the most revolutionary discoveries of the last fifty years is the demonstration that in the central nervous system of mammals neurogenesis is maintained in the adult life. In physiological conditions, neurogenesis is restricted to two defined germinal niches, represented by the lateral wall of the lateral ventricles and the subgranular layer of the hippocampal dentate gyrus. In humans, such areas display cellular organizations reminiscent of those found in rodents, suggesting the presence of neural stem cells supporting the generation of new neurons. The primary progenitors populating these niches exhibit stem cell features as they self-renew throughout life and are capable to give rise to the three neural lineages (astroglia, oligodendroglia and neurons). Surprisingly, they were found to display neuroanatomical traits of astrocytes, prompting a profound change in the traditional notion of glia, now conceived not only as metabolic and electric partner for neurons, but also as a potential source of progenitors. However, outside the germinal niches glial cytotogenic activity is normally repressed or restricted to gliogenesis. Yet, if properly implemented, it might be exploited *in situ* for reparative purposes, including possibly neuronal replacement. In the last years we have focussed our research on the cellular and molecular mechanisms of the astroglial reaction upon injury with the aims to i) disclose whether this process leads to astroglia de-differentiation and to the acquisition of a neural stem cell potential, and ii) identify targets for the development of novel therapeutic approaches.

In the adult intact parenchyma astrocytes are by large non-proliferative and essentially behave as quiescent postmitotic cells engaged in a highly specialised functional partnership with neurons and vessels. However, fate-mapping analysis of quiescent astrocytes showed that after traumatic lesion they acquire a stem cell phenotype and re-enter the cell cycle, being thus able to resume the precursor function of cell proliferation (Buffo et al., Proc Natl Acad Sci USA, 105:3581-6, 2008). Such cytotogenic response produces a subset of scar forming astroglia, yet no differentiation along distinct lineages occurs. Thus, despite their progenitor phenotype and proliferative response, reactive astroglia are not capable of displaying full germinal properties *in vivo* upon lesion. Yet, when the tagged cells were isolated from the injured gray matter and exposed to favourable *in vitro* conditions, at difference with those selected from the intact parenchyma they gave rise to self-renewing multipotent neurospheres generating astrocytes, oligodendrocytes and, most notably, neurons (Buffo et al., Proc Natl Acad Sci USA, 105:3581-6, 2008). Of note, this response is not a generalised feature of all glial cells, as oligodendrocyte progenitors fail to produce multipotent spheres upon lesion. Neurosphere forming cells can also be elicited from post-stroke cortical tissue in rodents and cells with progenitor traits have been recently described in human pathological material. Thus, quiescent astrocytes reacting to injury modify their biological status acquiring the potential of neural stem cells. Further support to this view is the evidence that proliferating glial cells reacting to damage can be engaged in neurogenesis *in vivo* upon forced overexpression of specific fate determinants governing neuronal differentiation during development (Buffo A et al, Proc Natl Acad Sci USA, 102:18183-8, 2005). However, these neurogenic attempts remain abortive, indicating the presence of restrictive factors in the damaged nervous tissue. On these bases, we propose that experimental manipulations able to promote in mature reactive astrocytes the reacquisition of stem/progenitor properties may stimulate effective reparative responses to neural damage.

## **Familiarity in entrapment neuropathies; toward the diagnosis of mucopolysaccharidosis**

EC Buffone, Ph La Marca , M Scarpa , M Campello

Neurology OC Bussolengo, Rehabilitation OC Malcesine, Department of Pediatrics, University of Padova, Italy; Neurosurgery OO.RR., Reggio Calabria, Italy

**Introduction** Mucopolysaccharidosis (MPS) represent a group of storage diseases due to lacking of lysosomal enzymes activity all along the glycosaminoglicans (GAG) catabolic pathway. According to different defects and the different residual enzyme activity, GAG accumulate and determine a wide spectrum of clinico-anatomical alterations affecting patients since their early childhood most of the times. One of the minor symptoms/signs is a carpal tunnel syndrome (CTS) which can occur in a familiar pattern when linked to a MPS disorder.

**Materials and Methods** Our contribution stems from a south-tyrolean family composed by parents and 3 daughters (all of them in their adulthood at diagnosis).

**Results** Mother and her daughters showed a bilateral median nerve compression. This unusual clinical manifestation compelled us to search for an underlying common root. First and third sibling had typical coarse facial features and other abnormalities that oriented towards a mucopolysaccharidosis diagnosis and finally a complete enzyme activities dosage brought to the diagnosis of MPS VI in a mild form due to a residual enzyme activity. A complete disease staging followed in order to set a starting point for the therapy (enzyme replacement therapy) which is still ongoing. After 6 months therapy some of the disease features are improving (e.g. CTS).

**Conclusions** An entrapment neuropathy like CTS can be a trivial problem but should always be framed in a general clinical picture which, rarely, belongs to a genetic disorder like MPS. ERT is promising but is still unknown how it will affect the disease's evolution during time

## **Comparative histopathology of skeletal and explanted heart muscles in a patient with laminopathy due to a novel *LMNA* gene mutation.**

E. Caldarazzo Ienco, V. Calsolaro, G. Ricci, L. Volpi, G. Ali, S. Benedetti, G. Lattanzi, M. Columbaro, P. Tanganelli, M. Emdin, G. Siciliano  
Department of Neuroscience; Department of Surgery, University of Pisa, Pisa, Italy  
Laboratory of Clinical Molecular Biology San Raffaele, Milano, Italy; IGM-CNR, Section of Bologna c/o IOR, Bologna, Italy; Department of Pathology, University of Siena; Department of Cardiovascular Medicine, G. Monasterio Foundation, CNR, Pisa, Italy

**Introduction** Mutations in the lamin A/C gene (*LMNA*) are known to be involved in several diseases such as Emery-Dreifuss muscular dystrophy (EDMD), limb-girdle muscular dystrophy type 1B (LGMD1B) and dilatative cardiomyopathies (DCM) with conduction disease, with considerable phenotype heterogeneity.

**Materials and Methods** We report the case of a 61-yr old caucasian female whose DNA analysis for *LMNA* gene revealed a novel frameshift 29 bp duplication in exon 6 of the *LMNA* gene (c.1102\_1130dup), responsible for a premature stop codon (p.Lys378ProfsX112) located in 2B coil of the putative protein. Family history was positive for an autosomal dominant DCM.

**Results** The patient presented with an adult-onset DCM phenotype later on associated with limb-girdle muscle involvement, undergoing cardiac transplantation at age 58. Skeletal muscle biopsy showed mild myopathic signs affecting both fiber types and slight increase in connective tissue; immunohistochemistry revealed only reduced emerin reactivity at nuclear level.

In explanted heart lamin A/C staining showed diffuse positivity with a certain reduction in some nuclear envelope districts; interestingly, the emerin staining resulted normal and properly localized at the nuclear rim in cardiomyocytes and endothelial cells but was completely absent in the interstitial cells nuclei.

**Conclusions** A secondary reduction of emerin muscle expression is a common finding in laminopathies, probably due to the direct interaction between emerin and lamin A. The selective defect of emerin appearance in interstitial cells of laminopathic heart of our case, similarly to a previous report by Morris and coll (2001) in the heart of an X-linked EDMD female carrier, rises the question on the molecular significance of the emerin involvement in these forms of myopathies.

## **REST/INRSF in normal and tumor nervous tissue and their cell lines**

V. Caldera, E. Cattaneo, L. Conti, L. Crisafulli, M. Mellai, D. Schiffer  
CNBO, Vercelli – General Hospital of Monza Foundation; University of Turin, Turin, Italy; Department Pharmacological Sciences, University of Milan, Milan, Italy

**Introduction** REST (Repressor element-1 transcription factor) contains a DNA-binding domain for the RE-1 binding site/neuron-restrictive silencer element (RE-1/NRSE) and two repressor domains. It is involved in many cell mechanisms and influences different target genes, depending on the context, and it is expressed ubiquitously in developing non neural tissues. Its activation or inactivation promotes or inhibits neuronal differentiation, so that it is not expressed in adult neurons, with some exceptions. REST is highly expressed in neural stem cells (NSCs) and its gene transcription is blocked as they exit cell cycle and differentiate.

**Materials and Methods** REST has been studied in formalin fixed and paraffin embedded samples of normal brains and in 81 operated gliomas (50 glioblastomas, 10 astrocytomas, 10 oligodendrogliomas, 5 medulloblastomas, 4 ependymomas, 12 meningiomas) by immunohistochemistry, immunofluorescence, Western blotting and Real Time PCR. The same procedures were applied to 12 glioblastoma cell lines.

**Results** In normal brains it is expressed in glial nuclei and in certain neurons of specific areas, mainly in Purkinje cells. In tumors all the nuclei were stained, with a greater intensity in hypercellular areas of glioblastomas with high vessel density and in anaplastic oligodendrogliomas. Neurospheres were intensely positive, whereas adherent cells were negative. Western blotting correlated with immunohistochemistry. Tumors with the highest expression of REST and neurospheres showed gene amplification.

**Conclusions** REST can be a marker of tumor stem cells or of a stem cell-like status, corresponding to dedifferentiated highly malignant clones after mutation accumulation which re-acquire stemness properties. It conditions the corresponding cell proliferation.

## **Expression of GAD isoforms and neuroactive amino acid levels in mouse brain areas: effects of pentylenetetrazole and minocycline.**

A. Cama, G. De Luca, E. Russo, M. Aguenouz, V. Macaione, PR. Calpona, R. Citraro, G. De Sarro, RM. Di Giorgio

Department Biochem Physiol & Nutr; Department Neurosci, Psych & Anest, University of Messina; Unit Pharmacol, University of Catanzaro, Italy

**Introduction** Pro-inflammatory and anti-inflammatory molecules are synthesized in glial cells during epileptic activity in those brain areas, where seizures initiate and spread. Minocycline (MIN), a semi-synthetic, second-generation tetracycline analogue, in addition to its own antibacterial properties, exerts neuroprotective effects in various experimental models. The neuroprotective role of MIN has not been investigated in animal models of epilepsy.

**Materials and Methods** In this study, we investigated whether MIN is neuroprotective against pentylenetetrazole (PTZ)-induced seizure in mice and measured the levels of some neuroactive amino acids by HPLC and the expression of GAD65 and GAD67 isoforms by Western blotting. MIN was able to antagonize PTZ-induced seizure with an ED<sub>50</sub> of 2.31 (1.25-4.27) mg/kg.

**Results** Administration of PTZ led to an increase of GABA and glutamate in the cortex and a reduction in the hippocampus. Instead, the administration of MIN alone increased GABA and glutamate in both areas. Both GAD isoforms were increased by MIN and unmodified by PTZ in most brain areas studied.

**Conclusion** MIN shows good anticonvulsant properties in this animal model and the increase in GAD65 might underlie this effect.

## **Mesenchymal stem cell treatment induces survival prolonging and symptom amelioration in a mouse model of amyotrophic lateral sclerosis.**

Capello E, Morando S, Milanese M., Principato MC, Giunti D, Caponnetto C, Vergani L, Mancardi GL, Bonanno G., Uccelli A.

Department of Neuroscience, Neurology Section, University of Genoa, Italy; Department of Experimental Medicine, Pharmacology and Toxicology Section, University of Genoa, Italy; Department of Biology, University of Genoa, Italy.

**Introduction** Mesenchymal stem cells (MSC), a subset of adult stem cells derived from the bone marrow stroma, have generated much enthusiasm as possible cell source for tissue repair including the nervous system. Recent studies have shown that MSC can also modulate immune responses and exert an anti-apoptotic effect on different cells including neurons. In addition, MSCs can migrate into the central nervous system when injected i.v.,

**Materials and Methods** thus we sought injecting i.v.  $10^6$  MSC in mice expressing mutant human superoxide dismutase (SOD1) with a Gly93Ala substitution [SOD1(+)-G93A(+)], a transgenic animal model of amyotrophic lateral sclerosis (ALS). Mesenchymal cells were injected at day 90, well after the symptom onset. Saline injected SOD1(+)-G93A(+) were used as controls. Control mice survive about 120 days.

**Results** MSC-treated mice exhibited a statistically significant prolonged survival time compared to saline injected controls. Such clinical effect was associated with a significant amelioration in the performance of behavioral motor tests in the MSC-treated animals. We have previously shown that glutamate exocytosis is enhanced in the spinal cord of SOD1(+)-G93A(+) mice, respect to controls. Interestingly, MSC treatment almost abolished this extra-release of the excitatory amino acid neurotransmitter. Upon i.v injection, a few luciferase-labeled MSCs were detected inside the mice spinal cord. Amelioration of some histological parameters were observed irrespective neural trans-differentiation.

**Conclusions** MSCs may be considered as an appealing therapeutic opportunity for ALS although the therapeutic effect does not rely on tissue repair.

## **Fusiform aneurysmal dilatation of the intracavernous carotid siphon in a case of neurofibromatosis type 1: simple coincidence?**

R. Conforti, A. Scutto, M. Cirillo, A. D'Amico, P. Candelaesi, R. Uccello, G. Lama, M.A.B. Melone

Department of Neurological Sciences and Department of Paediatrics, Second University of Naples; Department of Diagnostic Imaging and Radiotherapy Federico II University, Naples

**Introduction** Neurofibromatosis type I (NF1), is often mentioned among the heritable neurocutaneous disorders associated with stenoses or aneurysmal arterial disease affecting predominantly the renal arteries and less often the abdominal aorta (middle aortic syndrome), and mesenteric and peripheral arteries, but the association with intracranial aneurysms, has not been firmly established. Here, we report a case of multiple intracranial arterial aneurysms occurring in a patient affected by NF1.

**Materials and Methods** A 21 years-old man affected by NF1, was admitted to the hospital with a history of left painful ophthalmoplegia occurred one month before admission. A MR Imaging (MRI) of the brain with MR and CT Angiography, of the intracranial arteries was performed.

**Results** The patient was followed clinically since the age of 4 years, when a routine MRI has shown two intracranial aneurysms, one at left internal carotid artery as a huge fusiform intracavernous aneurysm and the second as contralateral smaller aneurysm. Otherwise in good health, the patient has followed a long clinical 18 year follow-up years, without any special treatment. At age 21 he suddenly manifested a left painful ophthalmoplegia. The MR Imaging (MRI), with MR and CT Angiography, showed a partial thrombosis of left intracavernous aneurysm. An oral anticoagulation quickly established has resulted in complete resolution of symptoms.

**Conclusions** Among the rare cerebrovascular abnormalities in NF-1, more than 85% cases are of purely occlusive or stenotic nature with intracranial aneurysm being uncommon. Including our case, we identified 29 cases of intracranial aneurysms in the literature. Predominantly, the aneurysms were located in the ICA circulation, being very rare multiple aneurysms, as evidenced in our patient. In conclusion, NF1, from presentation and diagnosis to its treatment, require continued attention, with emphasis on vascular disease and its management.

## **Rosuvastatin and Thapsigargin modulate $\gamma$ -secretase gene expression and APP processing in a human neuroglioma model.**

A. Crestini, P. Piscopo, M. Iazeolla, D. Albani, G. Forloni and A. Confaloni  
Department of Cellular Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy; Department of Neuroscience, M. Negri Institute for Pharmacological Research, Milan, Italy.

**Introduction** There are compelling evidences for an intimate association among an imbalance in brain lipid homeostasis, an intracellular calcium loss of homeostasis, an atypical neural apoptotic pathway and the sporadic form of Alzheimer's disease (AD). However, very little is known about the connection of these pathological mechanisms and the molecular process that produces the amyloid- $\beta$  peptides (A $\beta$ ). This study investigates whether the rosuvastatin, an HMG-CoA reductase, modulates the expression of genes involved in  $\gamma$ -secretase complex altering the A $\beta$  processing in a human cellular model for AD.

**Materials and Methods** We analyzed the statin effect combined with an apoptotic neurodegenerative *stimulus* induced by thapsigargin treatment that depletes intracellular calcium stores.

**Results** We found a differential transcriptional modulation of some  $\gamma$ -secretase cofactors relatively to the rosuvastatin treatment, which is dependent by the A $\beta$ -peptide concentration in the medium. Interestingly, the statin down-regulated the transcription of presenilin 1 and basigin genes in transgenic cultures, while in naïve control cultures, that do not produce A $\beta$  a gene modulation was obtained exclusively by the simultaneous administration of thapsigargin and statin. As regard to rosuvastatin, it neither affected the basal A $\beta$  levels nor counteracted APP processing or A $\beta$  over-production triggered by thapsigargin.

**Conclusions** Our results suggest that a direct effect of rosuvastatin on  $\gamma$ -secretase complex may alter gene expression, mantaining otherwise unaffected the enzyme activity.

## **Neurovascular brain dysplasias in Neurofibromatosis type 1 (NF1) patients : a Magnetic Resonance Angiographic (MRA) Study**

A. D'Amico, F. Caranci, F. D'Arco, A. Brunetti, D. Melis, R. Taurisano, E. Del Giudice, G. Lama, A. Scutto, R. Conforti and M.A.B. Melone

Department of Diagnostic Imaging and Radiotherapy, Department of Paediatrics Federico II University, Naples; Department of Neurological Sciences and Department of Paediatrics, Second University of Naples

**Introduction** Among the rare cerebrovascular abnormalities found in patients with NF-1, the most common is stenosis or occlusion of the cerebral arteries, which may have an appearance like the moyamoya disease. Cerebral aneurysms and arteriovenous fistulae are described as well, although less commonly.

**Materials and Methods** 80 patients with NF1 were studied with MR Imaging (MRI) of the brain and MRA of the intracranial arteries. Only 2 patients were submitted to an intracranial MRA with a 0.5 Tesla, while 61 were studied with a 1.5 Tesla machine. The remaining 17 brain MRI were accurately reviewed, searching vessel asymmetries. 2 patients were also submitted respectively to CTA and MRA of extracranial arteries, with intravenous contrast injection because a sub-occlusive narrowing of the intracranial internal carotid artery had been previously found. Finally a patient, examined by MRI presented multiple intracranial arterial aneurysms.

**Results** Our patients didn't show at MRI any ischemic lesion, being asymptomatic for such kind of pathology. Multiple intracranial arterial stenoses were found in 5 patients, respectively located: 1 at the carotid siphon, 2 at the supraclinoid carotid tracts, 2 involved the complete intracranial tract of the ICA, 4 the MCA, 3 the ACA, 2 the PCA, including a typical case of Moyamoya, showing a total of 5,64% of stenoses. We noted that ICA's stenoses were already well appreciable on brain MRI, while other localizations were only detectable with MRA. The patient with a huge fusiform intracavernous aneurysm at left internal carotid artery with a second contralateral smaller aneurysm

became symptomatic at 21 years old when suddenly manifested a left painful ophthalmoplegia caused by an aneurysm thrombosis better depicted by a subsequent MRA

**Conclusions** Vascular stenoses in NF1 population is reported in about 2,5 % of cases, probably underestimating the real incidence, because MR Angiography (MRA) isn't routine executed. Our studies suggest that the percentage of brain vascular stenoses in NF1 patients is more elevated than those in literature reported, and also we believe that intracranial aneurysms in patients with NF1 deserve special attention. For this reason we recommend the routinely use of MR Angiography for NF1 patients during MR Imaging of brain.

## **The age-related endocrine changes may play a role in physiological and pathological brain aging**

E. Ferrari and F. Magri

Department of Internal Medicine and Medical Therapy, Unit of Internal Medicine and Endocrinology, Foundation Salvatore Maugeri I.R.C.C.S., University of Pavia, Pavia, Italy

**Introduction** The neuroendocrine modifications occurring with aging on the whole seem to be related more to disorders of the relationships between neural and hormonal signals than to specific alterations of the various endocrine structures themselves.

The more relevant neuroendocrine modifications occurring with ageing affect melatonin and corticosteroids.

**Materials and Methods** This effect may be clearly appreciated throughout the study of their circadian rhythmicity, that we performed in sufficiently high groups of healthy elderly subjects and in elderly demented patients.

**Results** The circadian profile of plasma melatonin was clearly flattened in healthy elderly and even more in old demented patients.

Concerning the corticosteroid secretion, ageing is characterized by a selective impairment of androgens (DHEA and DHEA-s), whereas the cortisol secretion is relatively constant or even shows an age-related trends toward higher levels at evening and night-time.

**Conclusions** Due to the opposite effects of the two kinds of corticosteroids either in the periphery and in the CNS, the imbalance between androgens and glucocorticoids may be responsible for the occurrence in the CNS of a more neurotoxic steroidal milieu, already present in clinically healthy elderly subjects and especially in patients with senile dementia

## **Genetic variants affecting the expression of inflammation-linked genes and dementia in the very old: evidence from the Monzino 80-plus Study.**

G. Forloni, L. Polito, A. Signorini, S. Batelli, M. Tettamanti, U. Lucca, D. Albani  
Mario Negri Institute, Milan, Italy; Golgi-Cenci Research Center, Abbiategrasso  
Milan, Italy

**Introduction** Aging-associated dementia is a serious sanitary burden. Inflammation might contribute to dementia onset and progression. The predisposing role to dementia of single nucleotide polymorphisms (SNPs) in pro-inflammatory genes as interleukin-1 alpha (IL-1 $\alpha$ ), beta (IL-1 $\beta$ ) and tumor necrosis factor alpha (TNF- $\alpha$ ) is controversial and data about the frequency of these SNPs in the very old are required.

**Materials and Methods** Data were collected in a prospective, door-to-door population-based study of all eighty years or older residents in eight municipalities of Varese province, Italy (the Monzino 80-plus Study). Diagnosis of dementia was based on DSM-IV criteria. Participants that gave their consent for genetic screening ( $n = 672$ ) were evaluated for IL-1 $\alpha$  *rs1800587* (C/T), IL-1 $\beta$  *rs3087258* (C/T) and TNF- $\alpha$  *rs1799724* (C/T) SNP genotyping, performed by Restriction Fragment Length Polymorphism (RFLP).

**Results** The population examined had a mean age at baseline of  $89.0 \pm 4.4$  years and male:female proportion was 1:3.5. Around 36% of subjects were diagnosed as demented at baseline. In the non demented group, the IL-1 $\alpha$  T allele showed a frequency of 7.5%, while in the demented group had a frequency of 9.6 %. The IL-1 $\beta$  T-allele frequency was 10.2 % in non demented people and 12.9 % in the demented group. Finally, frequency of TNF- $\alpha$  T allele was 8.4 % and 7.8. % in the non demented and demented groups, respectively. None of these differences was statistically significant. A further comparison by sex stratification did not modify the results.

**Conclusions** Our genetic investigation found no evidence that in this very old Italian population dementia is associated to the genetic variability in three SNPs of IL-1 $\alpha$ , IL-1 $\beta$  and TNF- $\alpha$ .

## **Phenotype variability in FTD associated with Progranulin mutations.**

F. Frangipane, N. Smirne, R. Colao, L. Bernardi, G. Puccio, S.A.M. Curcio, M. Mirabelli, R. Maletta, M. Anfossi, M. Gallo, S. Geracitano, M.G. Muraca, A. Clodomiro, A. Borelli, R. Di Lorenzo, F. Comito, V. Valenti, S. Marzano, R.A. Leone, A.C. Bruni.

Regional Neurogenetic Center; U.O. of Microbiology and Virology, ASP-CZ, Italy

**Introduction** Frontotemporal Dementia (FTD) is a genetically and pathologically heterogeneous group of diseases. Mutations in the progranulin gene (PGRN) are responsible for familial forms of FTD. The aim of this study is to describe clinical phenotype of FTD associated to different PGRN mutations in an isolated calabrian population.

**Materials and methods** Fourteen FTD patients performed a complete neurological/neuropsychological evaluation. PGRN gene was sequenced in all subjects.

**Results** One known frameshift (1145insA in 10 related patients) and two novel missense (A266P and C126W, in 3 related patients and in one sporadic subject, respectively) PGRN mutations were identified.

All mutated patients presented at onset with behavioural FTD; the 1145insA carriers showed a dysexecutive profile: distractibility, deficit of planning, disinhibition, neurological examination evidenced extrapyramidal signs and primitive reflexes; the A266P carriers manifested apathy, reduction of verbal initiative (apathetic profile) and normal neurologic examination. The C126W carrier presented with paranoid delusions and irritability, normal neurologic examination.

Mean of age at onset was statistically different among 1145insA carriers (64.3yrs) and A266P carriers (76yrs).

**Conclusions** Our findings, related to different FTD phenotypes, seem to corroborate a genotype-phenotype relationship in PGRN mutations.

## **Beneficial effects of Phosphodiesterase 10 inhibition in the R6/2 mouse model of Huntington's disease**

F.R. Fusco, C.Giampà, D.Laurenti, S.Anzilotti, G. Bernardi  
Laboratory of Neuroanatomy, Santa Lucia Foundation IRCCS and Department of Neurosciences, University of Rome Tor Vergata, Rome, Italy

**Introduction** We have previously showed that TP10, a phosphodiesterase type 10 inhibitor, displays a neuroprotective effect in a rat quinolinic acid model of HD (Giampà et al, 2009). In this study, we sought to determine if TP10 exerts a neuroprotective effect in R6/2 mutant mice, which recapitulates, in many aspects, human HD (Mangiarini et al, 1996).

**Materials and Methods** Transgenic mice were treated with TP10 1.5 mg/Kg daily starting from 4 weeks of age. After transcordial perfusion, histological and immunohistochemical studies were performed.

**Results** We found that TP10- treated R6/2 mice survived longer and displayed less severe signs of neurological dysfunction than the vehicle treated ones. Primary outcome measures such as brain volume, striatal atrophy, size and morphology of striatal neurons, neuronal intranuclear inclusions and microglial reaction confirmed a neuroprotective effect of the compound. Moreover, TP10 treated R6/2 mice had a later onset and a lesser degree of severity of clasping behavior. TP10 was effective in increasing significantly the levels of activated CREB and of BDNF the striatal spiny neurons, which might account for the beneficial effects observed in this model.

**Conclusions** Our findings show that TP10 could be considered as a valid therapeutic approach for HD.

## **FUS/TLS genetic variability in sporadic Frontotemporal Lobar Degeneration**

D. Galimberti, C. Cantoni, C. Fenoglio, F. Cortini, E. Venturelli, C. Villa, F. Clerici, A. Marcone, L. Benussi, R. Ghidoni, S. Gallone, D. Scalabrini, M. Franceschi, S. Cappa, G. Binetti, C. Mariani, I. Rainero, M.T. Giordana, N. Bresolin, E. Scarpini IRCCS “Ospedale Maggiore” General Hospital; “Luigi Sacco” Hospital, University of Milan, Italy; San Raffaele Turro Hospital, San Raffaele Scientific Institute, Milan, Italy; Centro S.Giovanni di Dio-FBF, Brescia, Italy; University of Turin, Turin, Italy; Neurological Clinic, Nursing home Santa Maria di Castellanza, Varese, Italy

**Introduction** Recently, mutations in the fused in sarcoma/translated in liposarcoma gene (*FUS/TLS*) gene have been shown to be one of the cause of familial Amyotrophic Lateral Sclerosis (ALS). The FUS/TLS protein is 526 amino acids long and is encoded by 15 exons. It is a multifunctional protein, implicated in several steps of gene expression regulation including transcription, RNA splicing and transport, translation. Due to the well known similarities between Frontotemporal Dementia (FTD) and ALS covering genetic, clinical and pathological aspects a role of FUS in FTLN could be conceivable. Given these premises the main aim of the present study is to test genetic variability in the *FUS/TLS* gene for association with Frontotemporal Lobar Degeneration (FTLD).

**Materials and Methods** Two hundred and fifty one Italian patients with sporadic FTLD and 259 age-matched controls were tested for association with the tagging Single Nucleotide Polymorphisms (SNPs) rs741810 and rs1052352. Haploview 3.1 software was used to test for association.

**Results** Considering each SNP alone, no differences in either allelic or genotypic frequencies between patients and controls were found ( $P>0.05$ ), even stratifying according to gender or the presence of concomitant Motor Neuron Disease. Haplotype analysis failed to detect haplotypes associated with FTLD.

**Conclusions** According to these results, *FUS/TLS* does not act a susceptibility factor for the development of sporadic FTLD. Nevertheless, a confirmatory analysis is needed to conform these preliminary results.

## **An Alzheimer's disease patient with frontal phenotype linked to a novel PSEN1 mutation**

M. Gallo, N. Marcello, S.A.M. Curcio, R. Colao, G. Puccio, S. Geracitano, L. Bernardi, M. Anfossi, F. Frangipane, A. Clodomiro, M. Mirabelli, F. Vasso, N. Smirne, G. Muraca, R. DiLorenzo, O. Bugiani, G. Giaccone, R. Maletta, & A.C. Bruni

Regional Neurogenetic Center, Lamezia Terme, Catanzaro, Italy; U.O. Neurology, Arcispedale S. Maria Nuova, Reggio Emilia, Italy; Neurological National Institute "Carlo Besta", Milan, Italy

**Introduction** Presenilin-1 (PSEN1) mutations are causally associated with familial Alzheimer's disease (FAD) and its pathological features.

However, instances of Frontotemporal dementia have also been reported as caused by PSEN1 mutations.

**Materials and Methods** Patient underwent a standardized clinical, neuroradiological assessment. Tau, Amyloid Precursor Protein (APP), PSEN1, Presenilin-2 (PSEN2) genes were sequenced in proband. Neuropathological examination was carried out through standard stains and immunohistochemistry.

**Results** We identified a novel PSEN1 I143V mutation in a 55 year-old patient with personality changes, apathy. She manifested loss of speech, emotional unconcern, repetitiveness, wandering, temporal and spatial disorientation. At 68, she showed visual hallucinations; blurred language, *camptocormia* and rigidity. Family history revealed on four generation 13 subjects affected. Neuropathology evidenced severe atrophy in the frontal and temporal lobes. Amyloid and preamyloid A $\beta$  deposits were abundant in the neuropil of the cerebral cortex, mesial temporal structures, striatum, thalamus, brainstem and cerebellum. Numerous neurofibrillary tangles and neuropil threads were identified in the neocortex and in the mesial temporal structures. Neuronal loss and gliosis were evident.

**Conclusions** These data confirms that PSEN1 mutations may be associated with frontal phenotypes of AD and underlines the role of neuropathology in clarifying the correlations between clinical aspects and genotypic evidences.

## **Neurobiology of behavioural disorders in dementia**

P. Gareri, P. De Fazio, N.M. Marigliano, S. De Fazio, L. Gallelli, E. Russo, R. Citraro, G. De Sarro

Chair of Pharmacology and Chair of Psychiatry, Department of Experimental and Clinical Medicine, Faculty of Medicine and Surgery, University Magna Græcia of Catanzaro - Elderly Health Care Operative Unit, Catanzaro - Italy

**Introduction** Behavioural and psychological symptoms of dementia are a common feature of Alzheimer's disease (AD) and other dementias. Psychotic symptoms in AD patients are associated with genetic predisposition (i.e. presenilin 1, polymorphism of serotonin receptor genes, 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub>), different degrees of cell loss in CA1 and dorsal raphe nucleus. Other factors involved are differences in hemispheric size, blood flow and glucose metabolism.

**Materials and Methods** A Medline search was made using as key words dementia, behavioural disorders, neurobiology. Previous personal works were considered too.

**Results** Increased levels of norepinephrine are often associated to violent behaviour. Reduced levels of acetylcholine are not only related to cognitive symptoms but they may contribute to psychosis-agitation, apathy, indifference, disinhibition and aberrant motor behaviour. Neuronal losses in the nucleus basalis and a decrease in choline acetyltransferase are correlated with psychotic symptoms in Lewy body dementia. Decreased levels of serotonin play a pivotal role in aggression, agitation and impulsivity. Serotonin inhibits release of Ach from cortical and hippocampal cholinergic nerve terminals, possibly via 5-HT<sub>1B</sub> receptors in the hippocampus. The 5-HT<sub>3</sub> receptors may also inhibit the release of acetylcholine, whereas 5-HT<sub>1A</sub> receptors may mediate an increase in acetylcholine release. Increased levels of dopamine are also responsible for agitation and aggression, together with hallucinations and delusions. GABAergic dysfunction may be involved in behavioural disorders too.

**Conclusions** Changes in brain levels of some neurotransmitters seem to play a pivotal role in behavioural disorders in demented people.

## Differential expression of microRNAs in calpainopathies

A. Garufi, M. Aguenouz, O. Musumeci, G.L. Vita, N. Lanzano, A. Ciranni, C. Rodolico, A. Toscano, G. Vita.

Department of Neurosciences, Psychiatry and Anaesthesiology, University of Messina, Messina, Italy

**Introduction** Calpainopathies, a heterogenous group of neuromuscular disorders due to calpain-3 protein (CAPN3) deficiency, are due to: alteration on CAPN3 gene associated with a defined phenotype; alteration on dysferlin or other genes when calpain-3 protein deficiency is a secondary event; autocatalysis function of CAPN3 gene with normal protein quantity, but loss-of-function mutations cause its enzymatic inactivation. MicroRNAs (miRNAs) are small noncoding regulatory RNAs that reduce stability and/or translation of fully or partially sequence-complementary target mRNAs.

This study evaluates and quantifies the expression of specific skeletal muscle miRNAs in different groups of calpainopathies patients, to verify their role and involvement in the pathogenesis of diseases.

**Materials and Methods** Were selected 15 muscle biopsies CAPN3-deficient and divided into 3 groups: G1, 5 patients with primary deficiency (absence of CAPN3 with WB analysis and CAPN3 gene mutation); G2, 5 patients with primary deficiency (autocatalysis and CAPN3 gene mutation); G3, 5 patients with CAPN3 secondary deficiency and DYSF gene mutation; 4 normal controls. Total RNA was extracted from skeletal muscle biopsy and miRNAs were reverse transcribed with Megaplex miRNA reverse transcription kit (Applied Biosystems). Hybridization was done on a Human miRNA TaqMan Low Density Array panel containing 384 miRNA probes.

**Results** The results show that 98/384 miRNAs were overexpressed in CAPN3 deficiency (G1+G2+G3) vs CTR: 53 miRNAs were overexpressed in the primary deficit (G1 + G2) and 45 miRNAs were overexpressed in the secondary deficit (G3).

**Conclusions** All miRNAs expressed in 3 groups are involved in several pathways and their different expression suggests distinct pathogenic patterns. The presence of miRNAs involved in inflammatory and proteolytic processes in secondary deficiency of CAPN3 could be consequent to primary alteration (dysferlin deficiency).

## **Questionable pathogenic role of PSEN2 R71W mutation.**

S. Geracitano, M. Gallo, L. Parnetti, E. Mattucci, M. Anfossi, L. Bernardi, F. Comito, R. Colao, G. Puccio, F. Frangipane, S.A.M. Curcio, M. Mirabelli, C. Tomaino, F. Vasso, A. Clodomiro, R. DiLorenzo, N. Smirne, M.G. Muraca, T. Dattilo, R. Maletta, P. Calabresi, A.C. Bruni.

Regional Neurogenetic Center, ASP-CZ, Italy; Neurologic Clinic, University of Perugia, Italy

**Introduction** The clinical spectrum of rare presenilin-2 (PSEN2) mutations is characterized by an incomplete penetrance and variable clinical expression, overlapping with late onset Alzheimer Disease (AD).

**Materials and Methods** Patient underwent a standardized clinical and neuroradiological assessment. Amyloid Precursor Protein, PSEN1, PSEN2, Leucine-rich-repeat-kinase-2 genes were sequenced in the proband. DHPLC screening was performed in 100 cognitively healthy subjects.

**Results** We identified the PSEN2 R71W mutation in a patient with early onset AD and in two cognitively healthy subjects aged 57 and 70 years. At the age of 55 years, the patient showed decreased attention, flattened emotion, memory impairment, language reduction and apraxia. MMSE was 13/30,  $\beta$ -amyloid 1-42 CSF levels were 382pg/mL, total tau 421pg/mL and phosphorylated tau 70pg/mL, as typically seen in AD. Family history revealed that the patient's father was affected by AD and his mother and maternal grandfather were affected by Parkinson disease (PD).

**Conclusions** Our findings confirm the wide presentation of PSEN2 mutations regarding age at onset and variable penetrance. Onset was early in our AD case who showed family history for AD and PD, whereas an apparently sporadic late-onset case has already been published. However, the presence of R71W in two healthy subjects requires further investigations to elucidate the pathogenic role of this mutation.

## **Familial Alzheimer's disease associated with the A673V *APP* recessive mutation presents a distinctive neuropathological phenotype**

G. Giaccone, M. Botta, G. Di Fede, F. Moda, M. Catania, M. Morbin, F. Tagliavini  
IRCCS Foundation Neurological Institute Carlo Besta, Milan, Italy

**Introduction** Recently, the *APP* mutation A673V has been identified that stems out from all the other genetic defects inducing familial Alzheimer disease (AD), since it causes the disease only in the homozygous state [Di Fede et al, *Science* 323:1473, 2009]. The high interest raised by this report derives also by the observation that the heterozygous condition of this mutation seems to be protective from the disease.

**Materials and methods** We here provide an account on the neuropathological picture of the proband of this family, who recently came to death. This report is therefore the first to describe the neuropathology associated with a recessive mutation inducing familial AD.

**Results** Beside the marked severity of the lesions, peculiar features were the configuration of the A $\beta$  deposits that were often of large size, perivascular and exhibited a complete correspondence between the pattern elicited by amyloid stainings such as thioflavine S and the labelling obtained with immunoreagents specific for A $\beta$ 40 or A $\beta$ 42. Moreover, A $\beta$  deposition spared the neostriatum while deeply affected the cerebellum, and therefore was not in compliance with the hierarchical topographical sequence of involvement documented in sporadic AD.

**Conclusions** The neuropathological picture of familial AD associated with *APP* recessive mutation A673V presents distinctive characteristics compared to sporadic AD or familial AD inherited as a dominant trait.

## **Cellular localization of ERK in the R6/2 mouse model of Huntington's disease.**

C. Giampà, S. Anzilotti, L. Perrone, D. Laurenti, G. Bernardi, L. Colucci-D'Amato, M.A.B. Melone, F.R. Fusco

Neuroanatomy Laboratory, Santa Lucia Foundation IRCCS and Department of Neuroscience, Tor Vergata University Rome, Italy; Laboratoire de Neurobiologie des Interactions Cellulaires et Neurophysiopathologie, Marseille, France; Department of Neurological Sciences, II University of Naples, Italy

**Introduction** The mitogen-activated protein kinases (MAPKs superfamily comprises three major signaling pathways: the extracellular signal-regulated protein kinases (ERKs), the c-Jun N-terminal kinases or stress-activated protein kinases (JNKs/SAPKs) and the p38 family of kinases. ERK signaling has been implicated in a number of neurodegenerative disorders, including Huntington's disease (HD). Phosphorylation patterns of ERK and JNK are altered in cell models of HD. In this study, we aimed at studying the correlations between ERK and the neuronal vulnerability to HD degeneration in the R6/2 transgenic mouse model of HD.

**Materials and Methods** Immunohistochemistry for phospho-ERK (p-ERK, the activated form of ERK) and dual label immunofluorescence for p-ERK and each of the striatal neuronal markers were employed on perfusion-fixed brain sections from R6/2 and wild-type mice.

**Results** Our study shows that striatal neurons, both spiny projection and interneurons, are completely devoid of p-ERK immunoreactivity in the wild-type mouse. Conversely, parvalbumin-labeled GABAergic interneurons of the striatum are highly enriched in p-ERK in the R6/2 mice, cholinergic and somatostatinergic interneurons are devoid of it. Interestingly, the parvalbuminergic interneuron subpopulation of the striatum is the only interneuron subset that is extremely prone to degenerate in HD.

**Conclusions** Thus, our study confirms and extends the concept that the expression of phosphorylated ERK is related to neuronal vulnerability and is implicated in the pathophysiology of cell death in HD.

## **TDP-43 in circulating lymphomononuclear cells and cortex/spinal cord of patients with familial and sporadic amyotrophic lateral sclerosis (ALS): hints about the pathogenic mechanisms of aggregate deposition.**

S Grifoni, MT Rinaudo, A Chiò, G De Marco, A Pellerino, A. Naldi, A Calvo, MT Giordana

Department of Neuroscience and Department of Medicine and Experimental Oncology, University of Turin, Turin, Italy

**Introduction** TDP-43 is a highly conserved and ubiquitously expressed protein predominantly localized in the nucleus. In ALS tissue, it is delocalized in the cytoplasm and undergoes accumulation and aggregation. ALS cases bearing missense mutations in TARDBP coexpress the TDP-43 pathology of sporadic ALS.

**Materials and Methods** In the present study we analyzed by western immunoblotting the pattern of TDP-43 in the cytosolic (CF) and nuclear (NF) fractions of circulating lymphomonocytes (CLM) of ALS patients with and without TARDBP missense mutations and in spinal cord and frontal neocortex of patients with sporadic ALS.

**Results** In NFs from controls as well as ALS with TDP-43 mutation, TDP-43 protein was expressed in approximately comparable amount as a protein with a molecular mass of about 45 kDa. In CFs two protein bands sized around 43 kDa and 41 kDa were appreciable; the signal of the two bands was weak in controls, while either one or the other of the two bands prominently accumulated in the blots of ALS patients bearing TARDBP gene mutations; the accumulation of either the 43 or the 41kDa band was found also in some cases of ALS without mutation. The CFs blots from samples of spinal cord and frontal cortex of ALS patients without gene mutations showed a 45 kDa TDP protein not appreciable in normal tissue samples.

**Conclusions** The accumulation of one cytoplasmic form of TDP-43 in CLM is possibly linked to the gene mutation, presumably by impairment of mechanisms involved in the nuclear import-export systems. The same mechanism is apparently active also in some ALS cases without TDP gene mutation. The TDP band of higher molecular mass found in affected motor neurons of sporadic ALS is suggestive of an additional dysfunction occurring in motor neurons and contributing to the deposition of insoluble, ubiquitin-tagged protein aggregates in the cytoplasm. In conclusion the present study highlights a double pathogenic mechanism in ALS; in addition, an impairment of nuclear import-export system of TDP is also found in peripheral lymphocytes of some ALS cases without gene mutation. The relevance of the latter finding for treatment trials and monitoring remains to be clarified.

## Peripheral biomarkers research based on a case-control study

M. Iazeolla, P. Piscopo, A. Crestini, R. Rivabene, L. Malvezzi Campeggi, R. Di Fava, G. Talarico, M. Gasparini, A. Greco, L. Minghetti, N. Vanacore, G. Bruno, A. Confaloni

Department of Cell Biology and Neurosciences; National Center for Epidemiology Surveillance and Health Promotion, Istituto Superiore di Sanità, Rome; Department of Neurological Sciences, Memory Clinic, University of Rome "Sapienza", Rome, Italy

**Introduction** A blood test for early detection of Alzheimer's Disease (AD) risk is an imperative to develop preventive approaches and disease-modifying treatments. Biological markers can serve as indicators of presymptomatic biochemical change and then supportive for early screening. Classical biomarkers of AD, increased phosphorylated  $\tau$  and decreased  $A\beta$  in cerebrospinal fluids, are useful markers of neurodegeneration but more non-invasive cost-effective tools have remained elusive.

**Materials and methods** To this purpose we enrolled 142 subjects from the "Memory Clinic", University of Rome "Sapienza": 92 patients AD and 47 controls, correlated by sex and age. After obtaining informed consent whole blood was drawn from all subjects and plasma obtained by centrifugation. Plasmatic proteins analysis was performed by ELISA assays.

**Results** As biochemical markers, we studied the progranulin levels (PGRN),  $A\beta$ -42 peptide, plasmatic uric acid (PUA) and finally the anti-oxidant capacity (AOC). Moreover, all patients were genotyped for *APOE* as genetic marker.

**Conclusions** Even if the number of subjects involved is still rising, preliminary results indicate significative correlations between the analyzed parameters and Alzheimer' disease.

## **Neuropathologic findings in a patient with PSEN2 A85V mutation in the blood but not in the brain**

G. Marcon, P. Piscopo, A. Crestini, L. Malvezzi Campeggi, R. Rivabene, M.R. Piras, A. Confaloni, G. Giaccone, F Tagliavini

IRCCS Foundation Neurological Institute Carlo Besta, Milan, Italy; Istituto Superiore di Sanità, Rome, Italy; Neurological Clinic, University of Sassari, Italy

**Introduction** The A85V mutation in *PSEN2* gene has been described in an Italian family with several members affected by Alzheimer disease (AD). In the proband, the mutation was present in DNA samples obtained from blood and brain tissue, while in a cousin of the proband genetic analysis revealed the PS2 A85V mutation in DNA extracted from peripheral blood lymphocytes, but absence of this genetic defect in the DNA samples extracted from brain tissue and from fibroblasts. *Material and*  
**Materials and Methods** Neuropathological examination was performed in the two subjects.

**Results** The neuropathological scenario in the proband who presented a clinical picture of early onset dementia (age at death: 82 years) indicated a Lewy body variant of Alzheimer's disease (Braak VI of neurofibrillary changes and Lewy body pathology). At difference, the proband cousin (age at death: 82 years) showed diffuse neocortical A $\beta$  deposits and neurofibrillary changes with a topographic distribution indicating stage II of Braak. No Lewy bodies were observed.

**Conclusions** These findings indicate that the presence of a PS2 mutation in the blood but not in the brain influences the expression of the disease.

## **Complement C1-inhibitor (C1INH) alters the expression Alzheimer's amyloid-beta precursor protein induced by thrombin in human endothelial cells**

L. Mastronardi, B. Arosio, C. Vergani and L. Bergamaschini.

Geriatric Unit, IRCCS Foudation Ca' Granda Ospedale Maggiore-Policlinico, University of Milan, Milan, Italy

**Introduction** Thrombin is important in linking the activation of the coagulation pathways to inflammation in AD brain. Thrombin increases vascular permeability, potentiates the vasoactive effect of bradykinin, and regulates amyloid-beta precursor protein (APP) secretion from vascular endothelial cells, indicating that change in expression of APP during cellular stress or inflammation may contribute to A deposition in AD. Complement C1 inhibitor (C1INH) binds to endothelium potentiating its inhibitory effects on C1, FXIIa, FXIa, kallikrein, plasmin and thrombin. We tested the hypothesis that cell-bound C1INH could protects against APP expression and vascular permeability induced by thrombin on endothelial cells.

**Materials and Methods** Kinetic analysis of thrombin inhibition by CINH and C1INH bound to endothelial cells (HUVEC) by measuring the conversion of the chromogenic substrate S2366 and the variation of HUVEC permeability to albumin. The effect of C1INH on thrombin-dependent APP mRNA expression by RT-PCR

**Results** After incubation of thrombin and C1INH in a purified system, thrombin activity was reduced by 50%, and it was almost totally suppressed in the presence of HUVECs. Thrombin induces a two- to four fold increase in HUVEV permeability, it was reduced up two fold by C1INH. In HUVEC exposed to thrombin there was a 30% increase in APP mRNA expression which was prevented by C1INH.

**Conclusions** These findings indicate a novel role for Complement C1INH in inhibition of vascular endothelial activation. Since there is evidence of C1INH deficiency in AD brain, these observations could provide the basis for new therapeutic application of C1INH to target inflammatory processes in AD.

## **Significance of IDH1 and IDH2 mutations in gliomas and their correlations**

M. Mellai, O. Monzeglio, A. Piazzini, E. Andreoli, D. Schiffer  
CNBO, Vercelli – General Hospital of Monza Foundation, University of Turin, Turin,  
Italy

**Introduction** Somatic mutations of isocitrate dehydrogenase enzyme isoforms 1 (IDH1) and 2 (IDH2) have been recently described in a high percentage of astrocytomas and oligodendrogliomas. The two isoforms catalyze the conversion of isocitrate to  $\alpha$ -ketoglutarate with reduction of NADP<sup>+</sup>. Mutations are preferentially located in exon 4 of both IDH1 and IDH2 genes, affecting the aminoacid arginine at codon 132 (R132) of IDH1 gene and the homologous at codon 172 (R172) of IDH2 gene. Both sequences are involved in an evolutionary conserved region in the binding site of isocitrate, but it is not clear how these aminoacidic substitutions can influence the enzyme activity or whether depletion of  $\alpha$ -ketoglutarate or NADPH reducing equivalents can play a role as pro-oncogenic mutants.

**Materials and Methods** Screening of IDH1 and IDH2 mutations was performed in a series of 111 glioblastomas and of 50 low-grade gliomas (including 3 gemistocytic astrocytomas, 3 diffuse astrocytomas, 5 pilocytic astrocytomas, 1 anaplastic astrocytomas, 1 oligoastrocytomas and 21 grade II and 16 grade III oligodendrogliomas). Analysis was carried out on formalin fixed and paraffin embedded surgical samples by direct sequencing.

**Results** In low grade astrocytomas and oligodendrogliomas, IDH1 gene was found mutated in 50% of cases. R132H mutation represents the 96% of these cases, whereas R132C the 4%. In glioblastomas, R132H was found only in 2% of cases, whereas IDH2 gene was found mutated in only one glioblastoma (R172K).

**Conclusions** IDH1 and IDH2 mutations represent an early genetic event in gliomagenesis.

## **Anti-oxidant defence in plasma and cerebrospinal fluid in subjects affected by sporadic and genetic Creutzfeldt-Jakob disease**

L. Minghetti, R. Galeno, A. Greco, F. Cardone, M. Puopolo, N. Latronico, E. Baronio, A. Ladogana, M. Pocchiari

Department of Cell Biology and Neuroscience, Istituto Superiore di Sanità, Rome, Italy; Department of Anesthesiology-Intensive Care, Spedali Civili, Brescia, Italy.

**Introduction** Oxidative stress is a key factor in pathogenesis and progression of several neurodegenerative diseases, including Alzheimer's disease (AD) and Creutzfeldt-Jakob disease (CJD). The measurement of peripheral anti-oxidants represents a valid tool for evaluating the involvement of oxidative stress in the course of the disease. We have previously shown that peripheral anti-oxidant defences (or total anti-oxidant capacity, tAOC), are depleted in AD patients, and suggested that tAOC is a good index of the general anti-oxidant status of patients although it does not necessarily reflect the extent to which vulnerable neuronal populations are protected from oxidant processes (Minghetti et al, 2006).

**Materials and Methods** In the present study, we have measured the levels of tAOC in plasma and cerebrospinal fluid (CSF) samples from 18 non neurological patients (as control group), 27 sporadic and 13 genetic CJD patients. tAOC was measured by using a colorimetric assay based on the reduction of  $\text{Cu}^{++}$  to  $\text{Cu}^{+}$  by the activity of all anti-oxidant species present in the sample.

**Results** We found that the levels of AOC in both plasma and CSF were significantly lower in CJD patients than in the control group. Interestingly, CSF AOC was significantly lower in genetic than in sporadic CJD patients. Plasma/CSF AOC ratio in genetic CJD patients was significantly higher compared to both sporadic CJD and control groups.

**Conclusions** Our findings confirm the occurrence of oxidative stress in CJD and suggest that the genetic form might induce a more severe unbalance between the oxidative status of brain and periphery.

## **Alzheimer Disease (AD): anti-amyloidogenic properties of an A $\beta$ -protein variant form**

M. Morbin, G. Di Fede, L. Colombo, M. Salmona, A. Uggetti, G. Mazzoleni, F. Tagliavini

IRCCS Foundation Neurological Institute Carlo Besta, Milan, Italy

**Introduction** AD is the most common neurodegenerative dementia, it is usually sporadic, but about 5% of cases are familial with autosomal dominant inheritance. The current search for disease-modifying therapies has focused on amyloid  $\beta$  (A $\beta$ ), since a central pathological feature of AD is the accumulation of misfolded A $\beta$  in the form of oligomers and amyloid fibrils in the brain. According to this view altered A $\beta$  species are the prime cause of AD and the primary target for therapeutic intervention. We have recently identified an APP mutation resulting in alanine-to-valine substitution at position 673 corresponding to position 2 of A $\beta$ , that causes disease only in the homozygous state.

**Materials and methods** To gain information on the mechanisms of the recessive mutation in causing disease, we investigated the effects of the A673V variant on the aggregation and amyloidogenic properties of A $\beta$  using synthetic peptides homologous to residues 1-40 and 1-42 with and without the A-to-V substitution at position 2.

**Results** Laser light scattering (LLS), electron microscopy (EM), atomic force (AFM) and polarized-light microscopy after Congo red staining showed that the A673V mutation marked increases A $\beta$ 's tendency to aggregate and form amyloid fibrils. Surprisingly, the analyses of interaction between wt and mutated A $\beta$  peptides revealed that the peptide mixture built up far fewer amyloid fibrils not only than the mutated A $\beta$  but also than the wild-type A $\beta$  alone. Moreover, the aggregation rate of the peptide mixture was much lower and the aggregates were far more unstable than either the mutated A $\beta$  or wild-type A $\beta$  alone.

**Conclusions** On the basis of these results we generated a short synthetic peptide homologous to residues 1-6 of A $\beta$  carrying the A-to-V substitution and tested its ability to bind A $\beta$  fibrils and hinder amyloidogenesis. Polarized-light, EM and AFM showed that mutated A $\beta$ 1-6 inhibited the assembly of A $\beta$  into amyloid fibrils.

Thus the heterologous interaction between the mutated and wild-type A $\beta$  is favoured by the amino acid substitution at position 2 and affects nucleation or nucleation-dependent A $\beta$  polymerization or both, hindering amyloidogenesis.

## **Cell response to brain injury in Stroke-prone spontaneously hypertensive rats (SHRSP)**

E. Mura, A. Colonna, L. Cova, E. Zennaro, V. Silani, L. Sironi, P. Gelosa, N. El Assawy, M. Stramba-Badiale, G. Michailidis, F. Magrini, G. Busca, A. Zanchetti, A. Mauro

Auxologico Italian Institute, Verbania; Auxologico Italian Institute, Milano; Department of Pharmacological Sciences; Cardiovascular Medicine, University of Milan; University of Turin.

**Introduction** Salt-loaded SHRSP develop severe hypertension, cerebral oedema and proteinuria. Here we study the onset of ischemias and correlate the cellular response to brain injury with proteinuria and MRI analysis.

**Materials and methods** Salt-loaded SHRSP and Wistar-Kyoto rats (as controls) serially underwent cerebral MRI and control of proteinuria. At different proteinuria levels rats were sacrificed and divided in 3 experimental groups: Control, P1 (proteinuria>40mg/die) and P2 (proteinuria>100mg/die). Dissected brains were immunohistochemically processed to study glial reaction, oxidative stress, cellular death and neurogenesis.

**Results** With proteinuria over 100mg/die, cerebral MRI showed the presence of cortical ischemias, confirmed by the histological analysis. In the hippocampus and in the cortex we observed a strong glial reaction extending towards the contralateral hemisphere and following the corpus callosum. Close to lesions we found 4-hydroxynonenal- and TUNEL-positive cells, beside a wide nestin immunoreactivity. The statistical analysis on GFAP immunoreactivity demonstrated a correlation between the number of GFAP-positive cells and proteinuria. The comparison among the experimental groups showed an increase of the immunoreactivity in P2. The distribution of P2-GFAP cells follows a gradient which increases from the contralateral hemisphere towards the lesion.

**Conclusions** As expected close to cortical lesions there is cellular death (TUNEL+) together with oxidative stress (4-HNE+). In response to brain injury there is a strong glial reaction which precedes the onset of ischemias and strongly correlates with proteinuria levels. The presence of newly born nestin+ cells suggests the presence of a regional regenerative response.

## ***PMP22* expression levels in skin nerve fibers of Charcot-Marie-Tooth type 1A (CMT1A) patients: a biological marker of treatment efficacy**

L. Nobbio, D. Visigalli, R. Lombardi, M. Nolano, A. Solari, G. Lauria, L. Santoro, D. Pareyson, A. Schenone for the CMT-TRIAAL Group

Department of Neurosciences, Ophthalmology and Genetics and Center of Excellence for Biomedical Research, University of Genova, Italy; IRCCS Foundation C. Besta Neurological Institute, Milano, Italy; Department of Neurological Sciences, University of Napoli 'Federico II' and Neurology Division, 'S. Maugeri' Foundation, IRCCS, Telese Terme (BN), Italy

**Introduction** CMT1A neuropathy is due to a duplication of the peripheral myelin protein 22 (*PMP22*) gene. Therapeutic approaches to CMT1A should reduce *PMP22* levels. Chronic treatment of CMT1A mice with high dosage of ascorbic acid (AA) improves their phenotype by downregulating *PMP22*. A clinical trial using AA in CMT1A patients has been recently performed, using skin biopsies, among other outcome measures, as biological markers of efficacy

**Materials and Methods** We cloned the human sural nerve total *PMP22* cDNA into a plasmid to perform a titration curve, to quantify the real copy number of *PMP22* RNA in skin biopsies and eventually other tissues. Three *PMP22* transcripts have been described. The first, containing exon 1A, is specific of Schwann cells. The second, containing exon 1B, is ubiquitously expressed. The third one, lacking completely exon 1, has been rarely considered. AA seems to impact on *PMP22* expression levels through a negative regulation of the first transcript. Specific primers for the *PMP22* variants were then designed to perform relative quantification by RT-PCR on CMT1A skin biopsies.

**Results** The absolute quantification of total *PMP22* transcript in skin biopsies of CMT1A patients showed reduced levels of expression compared to the sural nerves but in a range right for the molecular purpose. Moreover, we found that all three variants are expressed in detectable manner in skin biopsies.

**Conclusions** A specific and sensible protocol to analyse *PMP22* mRNA expression in human skin biopsies has been established, which will allow to detect modulation of *PMP22* expression by pharmacological treatment.

## Genetic susceptibility factors in dementia

P. Piscopo, L. Bernardi, A. Adduci, M. Gallo L. Malvezzi-Campeggi, S. Geracitano G. Talarico, M. Anfossi, E. Conidi, N. Smirne, F. Vasso, R. Maletta, S.A.M. Curcio, M. Mirabelli, M. Menniti, A. Clodomiro, G. Bruno, F. Frangipane, M.R. Piras, G. Puccio, R. Colao, N. Vanacore, A. Confaloni and A.C. Bruni

Department of Cell Biology and Neurosciences, ISS, Rome, Italy; Regional Neurogenetic Center, ASP-CZ, Italy; Memory Clinic, Department of Neurological Science, University of Rome "Sapienza", Italy; Neurological Clinic, University of Sassari, Italy

**Introduction** Alzheimer's disease (AD) and Frontotemporal dementia (FTD) are genetically and pathologically heterogeneous neurodegenerative disorders. Despite the evidence that mutations in some known genes cause the diseases in families, etiopathogenesis of sporadic forms remains unclear. Several allelic variants in candidate genes have been evaluated as susceptibility factors. Our aim is to identify SNPs associated with risk and age at onset of AD and FTD.

**Materials and Methods** This is a case-control study in which 781 subjects including 415 AD and FTD patients (mean onset age  $78 \pm 9.7$ ) and 366 age/sex matched controls were enrolled. DNA was obtained after informed consent and genetic analysis was performed by PCR-RFLP or direct sequencing. SNPs analyzed were in genes associated to cholesterol (*APOE*, *Cyp46*) or A $\beta$  (*NCSTN*, *PEN2*, *PSEN1E318G*) metabolism, involved in the oxidative stress (*NOS3*) or in the phosphorylation of tau (*GAB2*) and in high LD with *APOE* (*TOMM40*).

**Results** Preliminary data showed different genotypic frequencies between patients and controls for *NCSTN* ( $p=0.01$ ) and *PEN2* ( $p=0.000$ ).

**Conclusions** Thus suggesting a possible role of these genes as dementia risk factors.

## **A patient with PSEN2 A85V mutation in blood but not in the brain**

P. Piscopo, G. Marcon, A. Crestini, L. Malvezzi Campeggi, R. Rivabene, M.R. Piras, G. Giaccone, F Tagliavini, A. Confaloni.

Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy; IRCCS Foundation Neurological Institute Carlo Besta, Milan, Italy; Neurological Clinic, University of Sassari, Italy

**Introduction** The A85V mutation in PSEN2 gene has been described in a Sardinian family with a clinico-pathological phenotype of Lewy body variant of Alzheimer's disease and inherited with autosomal dominant tract (Piscopo *et al* 2008). We found the mutation both in the blood and brain tissue of the proband. Successively, we also screened a cousin of the proband who died at age of 82 with clinical signs of dementia and presenting, at the onset, difficulties in behaviour, depression and an obsessive-compulsive syndrome.

**Materials and Methods** After obtaining informed consent, blood samples were taken from patient. Genomic DNA was extracted from peripheral blood leukocytes. Genetic analysis was performed using Direct sequencing by DNA sequencer Beckman CEQ 8000. Fibroblasts were obtained by skin biopsy and cultured under standard conditions.

**Results** The genetic analysis performed on peripheral blood lymphocytes, revealed the same aminoacidic change Ala→Val at codon 85. However, intriguingly, the successive sequencing performed also on the fibroblasts and the brain of the same subject was characterized by the absence of the allelic variant. Further molecular experiments performed on the specimens, confirmed their origin from the same subject.

**Conclusions** These findings may reflect a complex inter-tissue genetic heterogeneity, so far underestimated, also suggesting possible different mechanisms exerted by the mutation on the disease onset.

## **SORL1 is a risk factor for Alzheimer's disease in Italian population.**

P. Piscopo, G. Talarico, S. Barbati, M. Gasparini, L. Malvezzi Campeggi, E. Piacentini, M. R. Piras, G. Bruno, A. Confaloni.

Department of Cell Biology and Neurosciences, Istituto Superiore di Sanità, Rome, Italy; Memory Clinic, Department Neurological Science, University of Rome "Sapienza", Italy; Neurological Clinic, University of Sassari, Italy

**Introduction** Sortilin-related receptor 1 (SORL1) plays a key role in the recycling of the amyloid precursor protein (APP) from the cell surface via the endocytic pathways. In fact, the APP holoprotein is synthesized in the endoplasmic reticulum and Golgi; then, it is processed by  $\alpha$ - or  $\beta$ - secretase and, successively, by the  $\gamma$ -secretase complex generating A $\beta$  fragments. Alternatively, SORL1 binds APP holoprotein acting as a sorting receptor. Absence of SORL1 switches APP away from the retromer recycling pathway, directing it into the  $\beta$ -secretase pathway and so increasing A $\beta$ -peptide production. Recently, *Rogaeva* et al. described an association between some SORL1 genetic variants and late-onset Alzheimer's disease (AD).

**Materials and Methods** After obtaining informed consent, blood samples were taken from patients and control subjects. Genomic DNA was extracted from peripheral blood leukocytes. Genetic analysis was performed using Direct sequencing by DNA sequencer Beckman CEQ 8000.

**Results** The aim of this study was to verify if SORL1 could be a risk factor for AD analysing two populations from different Italian regions. The genetic analysis showed a different allelic distribution of SNP rs1010159 of SORL1 relative to controls in patients originated from *Latium* region, while no differences were found in isolated population of Sardinia Island.

**Conclusions** Our results support the evidence that genetic variants of SORL1 affect the susceptibility to develop AD, but, probably, its role as risk factor is depending on population ethnicity.

## **A case of familial corticobasal degeneration associated with a point mutation in amyloid beta-protein precursor (APP) gene**

G Piscoquito, A.M. Barbarulo, C. Coppola, G. Di Fede and R. Cotrufo  
First Neurological Clinic of Second University of Naples; Neurological Institute C. Besta, Milan, Italy.

**Introduction** Corticobasal degeneration (CBD) is a rare progressive neurodegenerative disease that typically presents with asymmetrical parkinsonism and cognitive dysfunction. In General CBD is a sporadic disease, although rare familial cases have been described.

**Materials and Methods** We describe the case of a 73-years-old man with positive familial history for cognitive impairment. He presented an insidious onset of apathy, social withdrawal and subsequently also deficit in making calculations, clothing apraxia, short-term memory impairment, space-time disorientation and progressive difficulty in using his left upper limb.

**Results** At neurological evaluation he showed severe asymmetrical pyramidal-extraparamidal rigidity, clothing apraxia, cortical sensory loss, bilateral grasping, Epstein sign and neglect the spontaneous use of the left upper limb; cognitive impairment was confirmed by neuropsychological assessment. CSF analysis revealed: beta amyloid 383 pg/mL (n.v. > 1200); total tau 229 pg/mL (n.v. <200); phosphorylated tau 32 pg/mL (nv <60). Brain MRI revealed significant cortical-subcortical distrectual atrophy. Brain PET showed a left-dominant hypometabolism in parietal and frontal areas. DaTSCAN showed a reduced asymmetrical uptake of the radiolabelled in striatum prevalent on the right. The genetic analysis showed a point mutation in the beta-protein coding region of the APP gene (Gly 708 mutation in exon 17); genetic analysis for protein tau and progranulin is still ongoing.

**Conclusions** The more frequent mutations described in familial CBD are in tau gene and in GNR. In our case there's a mutation in coding region of the APP gene which is silent at the protein level and to date no functional data is available.

## **Identification of genetic protective factors in CJD and healthy carriers of the E200K *PRNP* mutation.**

A. Poleggi, E. De Pascali, A. Ladogana, M. Puopolo, M. Pocchiari.  
Istituto Superiore di Sanità, Departmentt of “Cell Biology and Neurosciences”, Rome, Italy.

**Introduction** The Glu to Lys change at codon 200 (E200K) of the *PRNP* gene is a dominant mutation responsible for Creutzfeldt-Jakob disease (CJD) with highly variable expressivity and incomplete penetrance suggesting the existence of other modulating genetic factors. Thus, we performed GWA analyses on CJD patients and healthy relatives in the cluster area of Calabria.

**Materials and Methodos** GWA analyses was performed on 23 CJD patients and 51 healthy relatives (19 E200K carriers and 32 con carriers) from 15 pedigrees in order to identify possible genetic factors other than *PRNP* mutations or polymorphisms that modulate susceptibility and phenotypic variability in this prion disease.

**Results** We identified four SNPs (rs6692559, rs1064395, rs9793471, rs2057680) as candidate modifiers of the phenotypic expression or risk factors for the development of disease.

**Conclusions** These results indicate that genetic factors, other than *PRNP* gene, may modulate the onset and the progression of disease. These variants may regulate the conformational change of the prion protein or its metabolism and may be the basis for novel therapeutic strategies to delay the onset or prevent CJD in *PRNP* mutated carriers.

## **Sirtuin involvement in Environmental Enrichment paradigm in APP23 mice**

L. Polito, D. Albani, G. Forloni

Mario Negri, Milan, Italy; Golgi-Cenci Research Center, Abbiategrasso (MI), Italy

**Introduction** Sirtuins are proteins belonging to the histone deacetylase family, participate in a variety of cellular functions and might play a role in aging and age-associated diseases. Mammalian sirtuins are coded by 7 distinct genes (*SIRT1-SIRT7*) and recent data highlighted a possible role for this family in response to external stimuli such as calorie restriction and physical exercise. Environmental enrichment (EE) is a new paradigm that enhances brain activity and mitigates behavioural deficits induced by different pathological insults.

The aim of our study was to recapitulate EE benefits in a mouse model of AD (APP23) and to investigate if sirtuins could be involved in this paradigm.

**Materials and Methods** Mice (10 per group) were housed in standard housing conditions. After weaning, mice belonging to the enriched groups were moved to enriched cages for 5 months. Enriched cages were larger cages equipped with toys (balls, tunnels, igloos) that were renewed twice a week. At six month mice were tested with object recognition test. Then mice were sacrificed and sirtuin mRNA and protein levels were measured by Real Time PCR and Western Blotting respectively.

**Results** The behavioural test revealed a significant cognitive deficit in APP23. EE partially recovered the deficits in recognition memory. Moreover, EE led to changes in sirtuin expression profile.

**Conclusions** These preliminary data confirm that EE compensates behavioral deficits and could highlight an involvement of sirtuin protein family in modulating the outcome of EE.

## Gain of glycosylation as a possible mechanism of myelin protein zero mutations

V. Prada, M. Passalacqua, S. Scazzola, M. Bono , P. Luzzi, E. Bellone, G.L. Mancardi, P. Mandich, A. Schenone, M. Grandis.

Department of Neurosciences, Ophthalmology and Genetics; Department of Experimental Medicine, University of Genova, Genova, Italy.

**Introduction** Mutations in the gene *MPZ*, encoding for myelin protein zero (P0), the main protein of the peripheral nervous system, cause inherited neuropathies collectively called Charcot-Marie-Tooth 1B (CMT1B). We report the first case of missense mutation in *MPZ* causing a gain of glycosylation in P0.

**Materials and Methods** Clinical, electrophysiologic and neuropathological data were evaluated. The trafficking of the mutant protein and the pathomechanisms were characterized in both HeLa and rat schwannoma cells.

**Results** The patient presented with a severe neuropathy causing a delay in early milestones. A sural nerve biopsy revealed a remarkable loss of myelinated fibers with myelin outfoldings in the remaining ones. The genetic analysis of *MPZ* showed a missense mutation at aspartic acid 32 which was replaced by asparagine.

This mutation introduces a potential new glycosylation sequence. We confirmed that the mutant protein is hyperglycosylated and is partially retained into the Golgi in all our *in vitro* models. By alternatively deleting the endogenous (Asp32Asn+Tre95Met) or the new glycosylation sequence (Asp32Asn+Ser34Ala) we obtained mutant proteins normally trafficked to the cell membrane.

**Discussions** We describe a particularly severe *MPZ* mutation. Theoretically three pathomechanisms were possible: the change from an acidic aminoacid to a neutral one, a new glycosylation site in a wrong position, deranging the protein structure, or hyperglycosylation. By sequential experiments we provided proof of principle that hyperglycosylation is the main mechanism of this mutation.

Gain of glycosylation is not a rare mechanism of human mutations, but was never demonstrated in inherited neuropathies.

## **In vitro toxicity of three amyloidogenic variants of transthyretin (TTR).**

L. Pradotto, L. Vigna, G.E. Walker, A. Mauro

IRCCS Auxologico Italian Institute, Piancavallo (VB); University of Turin.

Amyloid deposition of transthyretin (TTR) variants is related to neurological diseases such as familial amyloidotic polyneuropathy (FAP). In FAP the TTR amyloid deposits are associated to loss of axons in the autonomic and spinal nerves and to neurons loss in the autonomic and spinal ganglia. Although apoptosis was postulated it's still unknown the mechanism of cell death and it is unclear if cytotoxic effects is are induced directly by amyloid deposit or by amyloid intermediates. The present study aimed to synthesize three recombinant mutant TTR proteins (V14D, V30M, L55S) and characterize their effects on two cell lines, the IMR-32 (human neuroblastoma) and RN22 (rat Schwann cell line).

## **BK virus encephalopathy in Good's syndrome: case report.**

G. Ricci, A. Chiti, M. Baldini, V. Pelliccia, D. Frosini, C. Pizzanelli, A. Servadio, I. Del Corso, G. Naccarato, G. Siciliano

Department of Neuroscience; Department of Surgery; Department of Oncology, University of Pisa, Pisa, Italy

**Introduction** BK polyomavirus (BKV) encephalopathy has been reported as very rare complication of acquired immunodeficiency condition.

**Materials and Methods** A 52 years-old female patient came to our attention for rapid and progressive cognitive impairment, apatia and ataxic gait. Two years before she had been diagnosed with acquired immunodeficiency associated with adult-onset thymoma (Good's syndrome) and had been subjected to thymectomy; then she had started therapy with subcutaneous Immunoglobulin.

**Results** Hematological and biochemical blood analysis confirmed CD4/CD8 ratio inversion and ipogammaglobulinemia, according to the previous diagnosis of Good's syndrome; urine exam revealed microematuria. Electroencephalography showed sporadic and diffuse delta-waves. Brain magnetic resonance disclosed bilateral asymmetric extensive hyperintense lesions in periventricular and subcortical white matter. Cerebral angiography was normal, excluding vasculities. Skin and muscle biopsy resulted unremarkable. Blood and cerebrospinal fluid RT-PCR for BKV/JCV and herpetic virus were negative. The stereotaxic cerebral biopsy was then performed. Brain histology showed high presence of glial cells and focal perivascular CD3+ T lymphocytes infiltration, possibly suggestive of differentiate astocytoma (II grade of the WHO classification). Subsequent RT-PCR for viral genoma detection resulted positive for BKV genoma and BKV associated encephalopathy was diagnosed. The patient further rapidly worsened, becoming bedridden; she died 10 months after neurological symptoms onset.

**Discussion:** In immunocompromised patients with neurologic symptoms, a diagnosis of BKV associated encephalopathy could be considered, even without evident urologic abnormalities. We report the first case of BKV encephalopathy in a patient affected by Good's syndrome.

## **Comparison of three different experimental rat glioma models, Fisher/F98, GS9L and Wistar C6: a MRI, PET and histological study**

F. Ronchetti, S. Valtorta, L. Politi, A. Lo Dico, N. El Assawy, S. Calderoni, AM Brioschi, GP Zara, F. Zenga, G. Scotti, RM Moresco, A. Mauro  
Auxologico Italian Institute, Piancavallo (VB); Nuclear Medicine and PET Cyclotron Centre Neuroradiology Department, San Raffaele Scientific Institute, University of Milan-Bicocca; IBFM-CNR, Milan, *Supported by a fellowship of the Doctorate School of Molecular Medicine, University of Milan, University of Turin, University of Brescia*

**Introduction** Experimental models of brain tumor have been largely used to evaluate the effects of new therapeutic approaches.

**Materials and Methods** Aiming to select an experimental glioma model useful to study the efficacy of new colloidal vectors for chemotherapy, we compared three well-known glioma models obtained in rats by intracerebral stereotactic implantation of glioma cells. Experimental models compared were: C6 glioma cells implanted in Wistar rats (allogenic), F98 glioma cells in Fisher rats (syngenic), and GS9L gliosarcoma cells in Fisher rats (syngenic). Tumor development was serially monitored by means of MR, PET ([F-18] FDG), and histological techniques, aiming to correlate morphological macroscopic features, metabolic activity, and microscopic characteristics. Analyzed parameters included tumor volumes, growth rates, invasiveness and infiltration features, angiogenesis, necrosis extension, perivascular infiltration, glial reaction, as well as cell proliferation, differentiation and apoptosis.

**Results** The three experimental glioma models showed important differences both in cell features and in growth characteristics. The syngenic Fisher/F98 model clearly displayed highest growth rates, associated with elevated proliferation indexes, invasiveness, large areas of necrosis, and extensive expression of nestin. On the contrary, growth rates of the allogenic Wistar/C6 glioma were inferior and, in some cases, probably influenced by extratumoral factors (immune?). The Fisher/GSL showed tumor cell features slightly different from those of the other two models, and a less invasive growth.

**Conclusions** Our study indicates that these experimental glioma models, despite large apparent similarities, shows significant differences in growth and cellular features. These differences must be carefully considered when planning experiments devoted to test the efficacy of new antineoplastic therapies.

## **Cognitive testing and correlation with cerebrospinal fluid Abeta, tau and Ptau levels in subjects with Mild Cognitive Impairment**

E. Rotondo, D. Galimberti, R. Vimercati, P. Corti, L. Bergamaschini, C. Fenoglio, M. Serpente, C. Cantoni, M. De Riz, N. Bresolin, C. Vergani, E. Scarpini  
University of Milan, IRCCS Foundation Ospedale Maggiore-Policlinico, Milan, Italy

**Introduction** To correlate neuropsychological testing with cerebrospinal fluid (CSF) Amyloid beta ( $A\beta$ ), tau and hyperphosphorylated (P)tau levels in patients with Mild Cognitive Impairment (MCI). Mini Mental State Examination (MMSE) is currently used to determine global cognitive state in subjects with MCI. However, cognitive domains that better predict progression from amnesic MCI to Alzheimer's disease (AD) are episodic verbal memory, evaluated with Recall Test (SRT) and Learning of Couples of Words (LCW), and executive functioning, tested by Coloured Progressive Matrices of Raven (CPMR) and Clock Drawing Test (CDT).

**Materials and Methods** Eighteen subjects with amnesic MCI were recruited. All of them underwent neuropsychological testing, including global cognitive assessment by MMSE, episodic verbal memory by SRT and LCW and executive functions by CPMR and CDT. Six out of 18 underwent lumbar puncture at time of diagnosis.  $A\beta$ , tau and Ptau were evaluated by ELISA. Statistical analysis was carried out by using t-test and Spearman test for correlations.

**Results** In the whole population, a significant positive correlation between SRT and MMSE was observed ( $\rho=0.577$ ;  $P=0.012$ ). In addition, a trend towards a positive correlation between CDT and MMSE was found ( $\rho=0.444$ ;  $P=0.064$ ). Considering CSF biomarker levels, three subjects showed an altered pattern and converted to AD after few months, whereas remainders had a normal profile and did not convert. Comparing these two groups, converters exhibited worse scores at LCW and CDT as compared with non-converters (8.66 versus 6.66, and 4.33 versus 3.33,  $P>0.05$ ).

**Conclusions** According to these results, SRT and CDT are likely more representative of episodic verbal memory and executive functions, respectively, and reflected more sensitively MMSE score. The same analysis in a small group of subjects in which CSF biomarkers predicted diagnosis, confirmed data obtained on CDT. Nevertheless, LCW, rather than SRT, seemed to be the more specific episodic verbal memory test to predict conversion from MCI to AD.

## **Neuropathological considerations by a case of Alpers-Huttenlocker disease**

D. Ruggeri, S. Galatioto

Emergency Surgery Unit - University of Messina –Messina Italy

**Introduction** Alpers' disease is a progressive degenerative disorder in infancy whose neuropathological microscopic changes mainly consist of severe widespread loss of cortical brain neurons together with marked spongy state and glial proliferation. Recently some researchers hypothesized the disease could be a disorder of mitochondrial machinery especially in the cases where the brain findings coexist with liver and/or heart severe damages.

**Materials and Methods** We report the pathological study of such a case

**Conclusions** We discuss the relationship between liver and brain damages as well the mitochondrial hypothesis.

## **Diagnostic validity of cytoplasmic expression of Major Histocompatibility Complex class I for human inflammatory myopathies diagnosis.**

R. Salaroli, E. Baldin, R. Rinaldi, V. Papa, L. Badiali De Giorgi, L. Tarantino, R. Meliconi, M. Fusconi, N. Malavolta, R. D'Alessandro, G. Cenacchi

Clinic Department of Radiological and Cytopathological Sciences; Department of Neurological Sciences; Department of Clinical Medicine, Alma Mater Studiorum University of Bologna, UO Neurology-Cirignotta; UO Pathology-Grigioni; UO Internal Medicine-Bianchi. Internal Medicine-Borghesi, University Hospital S.Orsola-Malpighi, Bologna, Italy

**Introduction** The inflammatory myopathies (IM) are an heterogeneous group of acquired disorders of skeletal muscle with undefined etiology and pathogenesis. Muscle biopsy is the gold standard for the diagnosis; the histological cornerstone is the identification of cellular infiltrates in muscle tissue, however infiltrates are not always present. Induction of Major Histocompatibility Complex class I (MHC-I) antigen in muscle fibres precedes inflammatory infiltrates, persists in chronic phase, and is unaffected by immunosuppressive therapy so it is considered a good marker of IM. Many Authors consider only the sarcolemmal MHC-I staining even if evidences that a reticular pattern of internal MHC-I reactivity in fibres of myositis patients are reported.

**Materials and Methods** MHC-I expression was detected, by an immunohistochemistry test, in 64 adult patients. Samples were scored by two independent and blinded investigators and an average of 580 fibres were evaluated for each biopsy. The percentage of MHC-I internal labelled fibres was determined and interobserver reproducibility was evaluated.

**Results** The positive muscle fibres displayed staining of the cytoplasm rather than of the sarcolemma. Positive fibres were observed in all samples (21 IM cases and 43 controls). Interobserver reproducibility was moderate ( $K=0,568$ ). The specificity of the test was of 100% when the percentage of the internal labelled fibres was higher than 50%, as mean of the two observers.

**Conclusions** This is the first study on the validity of internal MHC-I expression for the IM diagnosis. Although reproducibility should be improved the high specificity observed may be useful in the clinical practice .

## **Tumor stem cells and stem cell-like phenotype in malignant gliomas**

D. Schiffer, V. Caldera, M. Mellai, L. Annovazzi, M. Lanotte, P. Cassoni  
CNBO (Vercelli) – General Hospital of Monza Foundation; Department of Neuroscience/Neurosurgery; Department of Biomedical Sciences and Human Oncology, University of Turin, Turin, Italy

**Introduction** Tumor stem cells in malignant gliomas are generally considered to derive from transformed neural stem cells, but, alternatively, they may represent dedifferentiated tumor cells after mutation accumulation which acquire a stem cell-like status. They show neural stem cell properties and genetic alterations of proliferating tumor cells.

**Materials and Methods** Twenty cell lines from glioblastomas as neurospheres and adherent cells were cultivated and transplanted in nude mice. They were studied together with primary tumors by immunohistochemistry, fluorescence immunohistochemistry, molecular genetics and Western blotting for a series of genes/proteins including stem cell markers.

**Results** Neurospheres developed in four cases showing the same genetic alterations and protein expression of the phenotype contained in primary tumors. Prevalence of Nestin expression upon GFAP, together with CD133, Musashi.1 and high expression of SOX2 and REST were the main features. In primary tumors these corresponded to hypercellular proliferating areas with a high vessel density. Adherent cells were more differentiated and less proliferating.

**Conclusions** In the phenotype of primary tumors, glio-vascular niches with the same properties of neurospheres were present, the significance of which cannot be exhausted in the only reminiscence of glio-vascular niches of SVZ. Even though the tumorigenic cells of malignant gliomas seem to be the transformed neural stem cells, it is possible that tumor stem cells, responsible for growth and recurrence, are dedifferentiated cells with an embryonic regression to a stem cell-like status.

## **ORL1 rs1050283: association analysis and influence on miRNA 369-3p binding in patients with Alzheimer's disease**

M. Serpente, C. Fenoglio, F. Clerici, A. Marcone, L. Benussi, R. Ghidoni, S. Gallone, D. Scalabrini, C. Cantoni, S. Cappa, G. Binetti, M. Franceschi, I. Rainero, M.T. Giordana, C. Mariani, N. Bresolin, E. Scarpini, D. Galimberti  
University of Milan, Fondazione Ospedale Maggiore-Genral Hospital;Luigi Sacco Hospital, University of Milan, Milan, Italy; San Raffaele Turro Hospital, San Raffaele Scientific Institute, Milan, Italy; Centro S.Giovanni di Dio-FBF, Brescia, Italy; University of Turin, Italy; Neurological Clinic, Nursing Home Santa Maria di Castellanza (Varese), Italy

**Introduction** ORL1 gene encodes for the lectin type oxidised low density lipoprotein receptor 1 and is one of the candidate genes proposed to be associated with Alzheimer's disease (AD). rs1050283 is a SNP in the 3' untranslated region (UTR) of ORL1 and is located in a binding site of miRNA 369-3p.

The aims was 1) to carry out an association analysis of rs1050283 in patients with AD compared with controls 2) to investigate the possible functional role of rs1050283 by influencing the binding affinity for miRNA 369-3p.

**Materials and Methods** rs1050283 genotype was detected by allelic discrimination using TaqMan Technology in 393 patients with AD compared with 368 age-matched controls. mRNA from Polymorphic Mononuclear Cells (PBMC) was extracted from 20 AD patients and 20 controls with different genotypes.

mRNA 369-3p expression was tested by using a specific probe (Assay ID:000557).RNU48 was used as an endogenous control.

Fisher's exact test was used for association analysis whereas Sigma stat software was used to compare miRNA 369-3p expression levels according to different genotypes.

**Results** A statistically significant increased frequency of the ORL1 rs1050283 allele frequency was observed in patients compared with controls (46% versus 43%;  $P=0.023$ , OR: 1.14, 95%CI: 0.80-1.64). The protocol to evaluate the effect of rs1050283 on miRNA 369-3p has been set up and validated.

**Conclusions** The ORL1 rs1050283 polymorphism likely acts as a risk factor for sporadic AD, possibly influencing the expression level of the gene through a modulation of the binding affinity with miRNA 369-3p.

## Genetic heterogeneity in an Italian FTL D series.

S. Testi, R. Pantieri, M. Cruts, S. Ferrari, T. Cavallaro, G.M. Fabrizi  
Department of Neurological and Visual Sciences, University of Verona; Department of Neuroscience, Bologna; Neurodegenerative Brain Diseases Group, Department of Molecular Genetics, VIB, Antwerp.

**Introduction** Frontotemporal lobar degeneration (FTLD) encompasses a broad spectrum of genetically and neuropathologically heterogeneous clinical syndromes including behavioural variant of frontotemporal dementia (bvFTD), progressive nonfluent aphasia (PNFA) or semantic dementia (SD). It may also be associated with parkinsonism or motor neuron disease (MND). Five different FTLD-associated genes have been identified: microtubule-associated protein tau (*MAPT*), progranulin (*PGRN*), valosin-containing protein (*VCP*), chromatin modifying protein 2B (*CHMP2B*) and TAR DNA binding protein (*TARDBP*). Linkage analyses have also identified a locus on chromosome 9 (9p21.3-p13.3) associated with FTD-MND, in which the gene is not identified yet.

**Materials and Methods** 50 patients fulfilling clinical diagnostic criteria for FTLD, 12 of whom with familial recurrence of dementia, were investigated for mutations in known FTLD-associated genes. In this series 42 index patients have a clinical diagnosis of bvFTD, 6 PNFA and 2 FTD-MND. Genomic DNA of the patients was analysed by Denaturing High Performance Liquid Chromatography (DHPLC) and sequencing.

**Results** Detected mutations were as follows. *PGRN* p.Phe86SerfsX170, p.Thr272SerfsX10 and the novel p.His400ThrfsX12 mutations, respectively associated with familial bvFTD, bvFTD preceded by prolonged Mild Cognitive Impairment (amnestic type) and PNFA. *MAPT* IVS10+16 C>T was associated with familial bvFTD. Taking together, *PGRN* and *MAPT* account for approximately 33% of all familial FTLD cases (four out of twelve) in our series, in agreement with mutational rates reported in literature.

In a single family-pedigree with bvFTD and neuropathological picture of FTLD-U (Ub+, Tau-, TDP43+), not associated with mutations in known genes, microsatellite marker analysis suggested a chromosome 9 haplotype shared by affected family members.

**Conclusions** The report emphasizes the allelic and locus heterogeneity of FTLD.

## **Uncommon clinical phenotype in Italian patients with *FUS/TLS* mutations.**

N. Ticozzi, C. Gellera, S. Messina, L. Maderna, C. Morelli, B. Poletti, A. Ratti, F. Taroni, R.H. Brown Jr, J.E. Landers, V. Silani.

Department of Neurology and Laboratory of Neuroscience, “Dino Ferrari” Center, Università degli Studi di Milano – IRCCS Auxologico Italian Institute, Milan, Italy; Units of Genetics of Neurodegenerative and Metabolic Diseases, Fondazione IRCCS Neurological National Institute “Carlo Besta”, Milan 20133 Italy; Department of Neurology, University of Massachusetts Medical School, Worcester MA, USA.

**Introduction** Mutations in the *FUS/TLS* gene account for ~5% of familial ALS (FALS) and ~1% of sporadic ALS (SALS) cases. We recently screened two cohorts of FALS and SALS patients of Italian descent.

**Materials and Methods** We studied four individuals harboring *FUS/TLS* mutations (three p.R521C and one p.G156E). All patients underwent a thorough neurological and neurophysiological assessment. We additionally performed a neuroradiological evaluation of the brain and cervical spinal cord (two patients), a muscular biopsy (two patients), CSF analysis (one patient) and neuropsychological assessment (one patient).

**Results** The three patients with the p.R521C mutation presented with a symmetric proximal weakness of the limbs, with subsequent spreading to the distal segments. The patient with the p.G156E mutation had a bulbar onset and a precocious involvement of the axial muscles of the neck and trunk. During the course of the disease, the patient developed a progressive impairment of executive functions, apathy and inertia. All patients had minimal upper motor neuron involvement. The ENG/EMG examination was compatible with a diagnosis of ALS in all cases. The muscular biopsy showed a severe neurogenic atrophy in both cases studied. The neuroradiological exams and the CSF analysis were normal. The neuropsychological evaluation of the patient with the p.G156E mutation was suggestive of a frontal lobe dysfunction.

**Conclusions** Our study suggests that patients harboring *FUS/TLS* mutations share an uncommon clinical phenotype characterized by a symmetrical, proximal or axial onset, dominated by a lower motor neuron involvement. We also describe the first ALS-FTD patient with a *FUS/TLS* mutation.

## **Chromosome 9 and sporadic Frontotemporal Lobar Degeneration: *KIF24*, but not *UBAP1*, is a risk factor in Italian population**

E. Venturelli, C. Villa, C. Fenoglio, F. Clerici, A. Marcone, L. Benussi, R. Ghidoni, S. Gallone, D. Scalabrini, F. Cortini, S. Cappa, G. Binetti, M. Franceschi, I. Rainero, M.T. Giordana, C. Mariani, N. Bresolin, E. Scarpini, D. Galimberti  
Foundation Ospedale Maggiore-General Hospital; Luigi Sacco Hospital, University of Milan, Italy; San Raffaele Turro Hospital, San Raffaele Scientific Institute, Milan, Italy; Centro S. Giovanni di Dio-FBF, Brescia, Italy; University of Turin, Italy; Neurological Clinic, Nursing Home Santa Maria di Castellanza (Varese), Italy

**Introduction** Linkage analysis identified a region on chromosome 9p associated with Frontotemporal Lobar Degeneration (FTLD). A detailed analysis of candidate genes lying in this region demonstrated an association with Ubiquitin Associated Protein (UBAP)1.

**Materials and Methods** The distribution of three Single Nucleotide Polymorphism (SNPs) located in the chromosome 9 haplotype identified via linkage analysis, including UBAP1 rs7018487, KIF24 rs17350674 and rs10814083, has been determined in a population of 284 patients diagnosed with FTLD, including 245 with behavioural variant Frontotemporal dementia (bvFTD), 23 with Progressive Aphasia and 16 with Semantic Dementia, compared with 318 age-matched controls.

**Results** A statistically significant increased frequency of the KIF24 rs17350674 AA genotype was observed in patients compared with controls (7.4 versus 2.5%;  $P=0.0068$ , OR: 3.63, CI: 1.58-8.35). Considering each syndrome separately, similar results were obtained in bvFTD versus controls (7.7 versus 2.5%,  $P=0.005$ , OR: 3.26, CI: 1.40-7.57). Stratifying for gender, a statistically significant increased genotypic frequency was observed in female patients as compared with female controls (8.9 versus 2.5%,  $P=0.008$ , OR: 3.85, CI: 1.36-10.93). In-silico analysis predicted that the substitution from W to L caused by the rs17350674 affects protein function ( $P<0.05$ ).

**Conclusions** The KIF24 rs17350674 polymorphism likely acts as a risk factor for sporadic FTLD, but a replication study would be needed to confirm these preliminary findings.

## ***CHMP5* and *BAG1* are protective factors for sporadic Frontotemporal Lobar Degeneration**

C. Villa, E. Venturelli, C. Fenoglio, F. Clerici, A. Marcone, L. Benussi, R. Ghidoni, S. Gallone, D. Scalabrini, F. Cortini, M. Serpente, C. Cantoni, S. Cappa', G. Binetti, I. Rainero, M.T. Giordana, C. Mariani, N. Bresolin, E. Scarpini, D. Galimberti

Foundation Ospedale Maggiore-General Hospital; Luigi Sacco Hospital, University of Milan; San Raffaele Turro Hospital, San Raffaele Scientific Institute, Milan, Italy; Centro S.Giovanni di Dio-FBF, Brescia, Italy; University of Turin, Turin, Italy

**Introduction** Previous studies identified a region on chromosome 9p associated with Frontotemporal Lobar Degeneration (FTLD). In this region there is a functional candidate, named Chromatin-modifying protein 5 (*CHMP5*) and, in the same linkage disequilibrium block, lies an additional candidate gene, named BCL2-associated athanogene 1 (*BAG1*)

**Materials and Methods** The distribution of the two Single Nucleotide Polymorphism (SNPs), rs844239 in *CHMP5* and rs706118 in *BAG1* have been determined in a population of 291 patients diagnosed with FTLD, including 253 with behavioural variant Frontotemporal dementia (bvFTD), 24 with Progressive Aphasia and 14 with Semantic Dementia, compared with 314 age-matched controls.

**Results** A statistically significant decreased frequency of the *BAG1* rs706118 G allele was observed in patients compared with controls (16.7 versus 23.9%,  $P=0.005$ , OR: 0.62, CI: 0.45-0.87), as well as a decreased frequency of the GG genotype (1.4 versus 4.8%;  $P=0.018$ , OR: 0.28, CI: 0.09-0.85). A statistically significant decreased frequency of the *CHMP5* rs844239 AA genotype was observed in patients compared with controls.

**Conclusions** The *CHMP5* rs844239 and *BAG1* rs706118 polymorphisms likely act as protective factors for sporadic FTLD.

## **Ubiquitin–proteasome system impairment in limb-girdle muscular dystrophy type 2A**

L. Volpi, G. Alì, G. Ricci, V. Calsolaro, E. Caldarazzo Ienco, C. Nesti, L. Petrozzi, L. Pasquali, M. Mancuso, M. Fanin, C. Angelini, G. Fontanini, , G. Siciliano  
Department of Neuroscience; Department of Surgery, University of Pisa, Pisa, Italy;  
Department of Neuroscience, University of Padova, Padova, Italy

**Introduction** Limb-girdle muscular dystrophy type 2A (LGMD2A) is an autosomal-recessive disorder, caused by mutations in the calpain-3 gene (CAPN3).

Up to date, the exact pathogenetic mechanism which leads LGMD2A muscles undergo necrosis is not completely clear. Several theories have been taken into account so far, among which the hypothesis that a reduced capacity for normal protein turnover in deficient CAPN3 muscles can induce abnormal protein accumulation, leading to cellular toxicity and cell stress response, as revealed in mouse knockout models (C3KO). Furthermore, previous studies on C3KO mice demonstrated that calpain-3 is necessary for ubiquitination and that it acts upstream of the ubiquitination machinery.

**Materials and Methods** In order to assess skeletal muscle protein turnover mechanisms, we studied the ubiquitin–proteasome system (UPS) and the Akt/mTOR mediated protein synthesis pathway in 9 human LGMD2A muscle biopsies. In particular, we used antibodies against ubiquitin, proteasome-20S, Akt, mTOR. Mitochondrial respiratory chain enzymes activity was also assessed in 5 samples.

**Results** The immunohistochemical analysis for UPS revealed a reduced level of ubiquitinated proteins in LGMD2A compared to control biopsies. In patient's samples, some fibres were highly positive for the Akt and mTOR reactivity, which was totally absent in controls. A few biopsies presented also mitochondrial enzymes alterations.

**Conclusions** Our data confirm the reduced activity of the UPS in human LGMD2A muscle, suggesting that loss of proper protein turnover may represent an important pathological feature in calpainopathies.