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Abstracts

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Chair, Organizing and Local Scientific Committees: Marina Melone with collaboration of AINP&NC and AIRIC Board

Plenary Lectures

Thursday, May 24th

Ca^{2+}signaling alterations and neuronal degeneration: cause or consequence?

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A number of studies suggests that an alteration in Ca^{2+} homeostasis is a hallmark of neurodegenerative pathologies; in particular, defects in Ca^{2+} homeostasis represent key aspects in determining the degeneration of neurons occurring in a variety of pathologies, e.g., stroke, Huntington's, Parkinson's and Alzheimer's diseases, amyotrophic lateral sclerosis and demyelinating diseases. In my presentation I will focus on cellular and animal models of the most common neurodegenerative disease in humans, Alzheimer's disease (AD). The vast majority of AD cases (over 95%) is sporadic, and the pathogenic mechanisms are still mysterious; in contrast, less than 5% of AD cases run in families, hence the name familial Alzheimer's disease (FAD), but, in these cases, the causal link between the disease and the gene mutations is firmly established and it is due to autosomal dominant mutations in three genes coding for amyloid precursor protein (APP) and presenilins (PS1 and PS2). The mechanistic link between the mutations and the early onset of neurodegeneration is, however, still debated. Most available data suggest that both FAD-linked PS mutants and endogenous PSs are involved in cellular Ca^{2+} homeostasis. The key question is whether alterations in Ca^{2+} homeostasis are the primary cause of neuronal dysfunction, are secondary to other molecular defects (especially A production), or are simply concomitant events that exacerbate the disease. In the present study I will discuss the approach we have pursued over the last years, that takes advantage of different Ca^{2+} imaging techniques to monitor in cell lines, primary neuronal cultures and acute brain slices, Ca^{2+} homeostasis at the cytoplasmic and organellar level. I will propose a model that integrates into a unifying hypothesis the contradictory effects on Ca^{2+} homeostasis of different PS mutations and I will discuss the relevance of these findings in AD and other neurodegenerative diseases.

Friday, May 25th

Frontotemporal lobar degenerations: new molecules, new ideas

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The term frontotemporal lobar degeneration (FTLD) is used to describe a heterogeneous group of neurodegenerative disorders, which neuropathologically is characterized by degeneration, predominantly affecting the frontal and temporal lobes. Clinically FTLDs present with distinct yet overlapping syndromes, of which behavioral variant frontotemporal dementia, progressive nonfluent aphasia and semantic dementia are the best-characterized. FTLD and amyotrophic lateral sclerosis (ALS) form a clinical and neuropathological disease (FTLD-ALS) spectrum, indicating common underlying disease mechanisms. Although the majority of FTLDs are sporadic, familial forms of the disease are common and the genes most frequently implicated are the microtubule-associated protein τ (MAPT), progranulin (GRN) and C9orf72 genes. Mutations of the valosin containing protein (VCP) and charged multivescicular body protein 2B (CHMP2B) genes are much less common, while those of the TARDBP and FUS genes, encoding the TAR DNA-binding protein-43 (TDP-43) and fused in sarcoma (FUS) protein, respectively, occur mostly in the ALS end of the FTLD-ALS spectrum. The current widely accepted classification subdivides FTLDs on the basis of the presence of one of a number of pathological proteins identified in the inclusion bodies observed postmortem. These disease-associated proteins forming inclusions are suspected to be closely associated with the underlying pathogenic process and the proteins responsible for the majority of the cases are τ, TDP-43

and the FUS protein. This presentation will focus on the current understanding of the molecular basis of the major subtypes of FTLD.

Workshop 1 "Pathogenetic mechanisms of brain tumors"

Thursday, May 24th

Role of chemokines in glioblastoma development: regulation of cancer stem cell survival

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The CXCL12/CXCR4 chemokine system has been involved in development, progression, invasion, metastasization, and neoangiogenesis in different human tumors. We reported that CXCL12 is an autocrine/paracrine factor that activate ERK1/2 and Akt to control proliferation and migration of glioblastoma established cell lines or primary cultures of human postsurgical glioblastoma cells. A recent carcinogenesis theory proposed that tumors are originated by rare cancer stem cells (CSCs) endowed with self-renewal properties and displaying high resistance to radio- and chemo-therapy that cause treatment failure, tumor relapse and metastasis formation. Using culturing protocols developed for neural stem cells, it is possible to isolate and grow in vitro cultures highly enriched in glioblastoma CSCs. Importantly, proteomic analysis of CSC cultures in comparison with established cell lines demonstrated that only the former retain a pattern of protein expression identifiable in the original tumors, while cell lines, likely due to indefinite culture passages, largely diverged. Thus, we were aimed to verify whether the role of CXCL12/CXCR4 axis identified in cell lines could be reproduced in human glioblastoma CSC cultures. We demonstrated that CSCs express both CXCL12 and CXCR4, and release the chemokine in vitro, although individual variations were observed among tumors. Surprisingly, at odd with glioblastoma cell lines, CXCL12 did not induce significant CSC proliferation, but exerted a self-renewal effect (as for spherogenesis assay), mainly affecting cell survival through Akt activation. These data suggest that CXCL12/CXCR4 are relevant factors in CSC biology, although significant differences can be observed analyzing cell lines or more reliable models, as CSC. (Supported by AIRC grant #IG9089 to TF).

Molecular pathology of pediatric gliomas

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High grade gliomas (HGG) (i.e. anaplastic astrocytoma WHO Grade III and glioblastoma WHO Grade IV) account for the 15% of all pediatric brain tumors and have a 3-year survival of less than 20% and high morbidity. Pediatric HGG are histologically indistinguishable from adult counterpart and the WHO classification does not provide any distinction between the two groups. However, recent investigations indicate that differences occur at the molecular level, and that therefore the molecular avenues to gliomagenesis in childhood are distinct to those encountered in adults. Study based on high-resolution analysis of copy number and gene expression signatures has demonstrated that pediatric and adult HGGs represent a related spectrum of disease distinguished by differences in the frequency of copy number changes, in specific gene expression signatures, and by the absence of IDH1 hotspot mutations. In pediatric HGG, numerous genes within the p53, PI3K/RTK, and RB pathways are targeted by focal gain or loss but with the exception of PDGFRA and CDKN2A, other alterations were found only at low frequency. Moreover, two very recent studies based on exome sequencing found somatic mutations in the H3.3-ATRX-DAXX chromatin remodelling pathway highly specific to pediatric GBM. Furthermore, the presence of H3F3A/ATRX-DAXX/TP53 mutations was strongly associated with alternative lengthening of telomeres (ALT) and specific gene expression profiles. These observations have highlighted the role that defects of the chromatin architecture plays in pediatric and young adult GBM pathogenesis. BRAF-KIAA1549 fusion gene is considered a driver genetic event in pilocytic astrocytomas (PAs). However recent studies have shown the occurrence of this fusion gene in diffuse gliomas of adults and also in pediatric HGG. This observation suggests that in a small percentage of pHGG, such deregulation might be related to 7q34 rearrangements, resulting in a novel in-frame KIAA1549-BRAF fusion gene like that found in PAs. However, the clinical significance of BRAF alterations in pHGG remains unclear.

Molecular mechanisms of medulloblastoma stem cell control by the morphogen hedgehog

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Sonic hedgehog (Hh) controls behavior of both neural stem cells NSC and cancer stem cells (CSCs) that have been identified in medulloblastoma (MB). The CSCs niches in MB can at least in part explain the resistance to conventional chemotherapy and the high rate of progression/relapse of these tumors. Aim of the study is to provide new insight in the molecular regulatory circuits involved in maintenance of stemness with particular regard to microRNAs as key molecules in gene expression regulation. To this end high-throughput miRNAs profiles have been carried out in: NSCs, differentiated NSCs and MB-CSCs. NSCs were isolated from wild type mice neonatal cerebellum, CSCs were isolated from both MB arised in Ptc+/– mice and human MB. Each sample group was analyzed in triplicate. MicroRNA levels of expression, obtained from NSCs, were compared to either differentiated NSCs or CSCs. Statistical paired t-test. A p value of less than 0.05 was considered as statistically significant. miRNA profiling in NSCs versus differentiated NSCs has

revealed specific pattern of microRNAs expression correlated to different Sonic hedgehog signaling activation context. On the other side we were able to determine microRNA features unique to both normal and cancer stem cells implicated in the establishment and/or maintenance of stemness. In fact miRNAs differentially expressed in cancer stem cells versus normal stem cells could be those required only in cancers stem cells potentially necessary to neoplastic transformation. The results of this study provide a broad overview of NSC and CSCs microRNAs.

Diagnostic, prognostic and predictive factors in intracranial ependymomas: from histological diagnosis to molecular profiles

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Ependymoma accounts for 3 – 6% of all CNS tumors. The incidence is higher in children and young adults where it is the second most common malignant brain tumor. In adults the most common location is the spinal cord, while in children ependymomas occur more commonly intracranially within the posterior fossa. Histologically, ependymomas are classified into 3 major subtypes: myxopapillary ependymoma (WHO Grade I), ependymoma (WHO Grade II), and anaplastic ependymoma (WHO Grade III). Histopathological grading between Grades II and III is often difficult and shows frequent disagreement among neuropathologists. Moreover, recent data suggest that these tumors display biological characteristics according to their location, therefore myxopapillary ependymomas and subependymomas should be considered clinicopathologic entities distinct from the other ependymomas. At the present, histopathologic parameters are not satisfying as prognostic factors and do not contribute to understand the clinical outcome of patients with ependymomas. Since current clinicopathological classification criteria, in particular for paediatric intracranial ependymoma, are inconsistent, the introduction of novel prognostic markers for therapeutic stratification is an important condition of future clinical management of patients. Only a few data are available concerning tumor-specific molecular abnormalities that could predict clinical behavior. In recent years, a number of gene expression profiling studies have been conducted on whole tumor tissue, to identify genes differentially related to ependymoma biology and oncogenesis. Furthermore, hypermethylation correlated with a decrease in expression of a number of tumor suppressor genes and pathways, could be playing an important role in tumor pathogenesis. However, the development of novel prognostic and predictive molecular biomarkers and targeted therapies is still required in order to enhance patient outcome.

Cytogenesis and cell lines in the understanding of the origin of the 2 glioblastoma types

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Primary and secondary glioblastomas (pGBM and sGBM) show different biological features and behave differently in relation with some markers: MGMT methylation, IDH1-2 mutations, EGFR amplification, TP53 mutations, etc. The problem is to discover the difference in the phenotypical or molecular pathways leading to each of them. Material and methods: Our experience is based on immunohistochemistry and molecular genetics of 200 GBMs, mostly pGBM, and of 21 GMB cell lines. Results: Our reasoning is based on the following findings: 1) It is much easier to obtain neurospheres in culture from primary than secondary GBM. 2) Neurospheres express the same stemness antigens as neural stem cells (NSCs) and the same genetic alterations of primary tumors. 3) Adherent cells do not show the same genetic alterations and express differentiation antigens. 4) IDH1-2 mutations are typical of sGBM and do not occur in pGBM. 5) There is a difference in MGMT methylation between pGBM and sGBM. 6) sGBM usually develop through the intermediate stage of anaplastic astrocytoma. 7) Both GBMs are supposed to develop from NSCs of subventricular zone (SVZ), but are located in the hemispheres far from it. Conclusions: It can be true that the tumors develop from the SVZ following the asymmetric division of primitive cells, their migration, the differentiation and mutation accumulation which conditions anaplasia and full malignancy. Process is the same in the two tumors, but its velocity is different. In sGBM it is slower allowing astrocytomas to develop first and then to transform. In pGBM the process is accelerated. Stemness is a condition preserved in pGBM from NSCs and lost in sGBM where, however, it can be reacquired with dedifferentiation.

Workshop 2 “Neurodegenerative Diseases”

Thursday, May 24th

Mechanisms of synaptic plasticity and neurodegeneration in Parkinson’s disease

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Parkinson’s disease (PD) is a neurodegenerative disorder characterized by a loss of dopaminergic neurons of the substantia nigra compacta resulting in decrease of striatal dopamine (DA) concentration and presence of distinctive α-synuclein (α-syn) inclusions. The α-syn inclusions are considered the pathological hallmark of PD. Recent studies highlight the role of α-syn inclusions in synaptic transmission and DAergic neuron physiology. Different α-syn transgenic mice show different levels of DAergic alterations, morphological and physiological age-dependent changes and may allow to assess specific aspects of PD pathogenesis

and role of striatal synaptic plasticity. Although a critical role of endogenous DA in the formation of striatal long-term potentiation (LTP) in MSNs has been demonstrated the question whether differential levels of DA denervation alter the activity and plasticity of striatal medium spiny neurons (MSNs) and specific interneurons is still open. We have characterized the effect of distinct levels of DA denervation on synaptic plasticity in both MSNs and striatal interneurons in toxic and transgenic α-syn models of PD. Current therapies are primarily based on pharmacological DA replacement through administration of DA precursor L-DOPA. However, L-DOPA treatment can result in side effects related to PD progression. In advanced PD, the vast majority of patients experience dyskinesias in response to medication. We have also demonstrated that in experimental PD dyskinesias are correlated to a maladaptive form of synaptic plasticity such as lack of reversal of previously-induced LTP. We suggest new strategies to prevent the induction of dyskinesia.

Autophagy in neurodegeneration: too less or too much?

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Production of mutated aggregate-prone proteins and mitochondrial dysfunction are common features of many neurodegenerative diseases, and it has been suggested that oxidative stress causes neuronal cell death through enhancing the accumulation of protein aggregates and of oxidized mitochondria. Macroautophagy (simply, autophagy) is a cellular homeostatic process in charge of the elimination of toxic protein aggregates as well as of redundant and damaged organelles (including mitochondria). This material is initially entrapped within newly formed autophagosomal vesicles which then fuse with lysosomes, wherein the material is degraded by cathepsins and other acid hydrolases. Elevated levels of autophagic vacuoles have been observed in *post-mortem* brain tissues of affected patients and animal models of Alzheimer's, Parkinson's and Huntington's diseases, indicating that autophagy is dysregulated in neurodegenerative diseases. Experimental data in animal models of Huntington's disease, Amyotrophic Lateral Sclerosis and Familial Neurohypophyseal Diabetes Insipidus indicate that stimulation of basal autophagy is beneficial to neurons through the removal of protein aggregates and oxidized mitochondria. Conversely, inhibition of autophagy activated by metamphetamine intoxication in dopaminergic neurons precipitates apoptosis. Recent data, however, also show that sustained up-regulation of autophagy, beyond the need to remove toxic aggregates and oxidized mitochondria, may be detrimental for neuronal survival. Whether and how autophagy contrasts or contributes to neurodegeneration depends on a delicate balance that is greatly affected by environmental stresses and the autophagy signaling pathway activated.

The complex network of interactions around amyloid precursor protein: possible role in neurodegeneration

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Amyloid precursor protein (AβPP) is a Type I receptor genetically involved in the genesis of Alzheimer's disease (AD). Mutations and/or overexpression of AβPP cause a pathogenic phenotype with neurodegeneration, amyloid (Aβ) deposits, gliosis and neurofibrillary tangles (NFTs). It is debated whether Aβ represents the primary cause of neuronal death or rather a disease's consequence [1]. There is even more controversy on the role of APP's processing by presenilin's (PSs) as "gain of function" (forming more Aβ) or rather a "loss of function" (forming aberrant Aβ or disrupting a yet unclear parallel function of PSs) [1]. In this regard, it is also interesting to note that many PSs substrates are type I receptors clearly involved in oncogenic cell proliferation (Notch, CD44, ErbB4, insulin-like growth factor 1 receptor etc.) [2]. Even the physiological function of AβPP is unclear, although many APP's interacting proteins with a clear relevance in signal transduction mechanisms suggest a likely role in cell motility and cell proliferation. In this talk I will focus the attention on AβPP as cell surface receptor and on AβPP's interacting proteins, describing different pathways putatively interested by AβPP's signaling and the consequences for amyloid and NFTs formation.

References

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Otx2 controls neuron subtype identity in ventral tegmental area (VTA) and antagonizes vulnerability to Parkinsonian toxin MPTP

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Mesencephalic-diencephalic dopaminergic neurons control locomotor activity and emotion and are affected in neurodegenerative and psychiatric diseases. The homeoprotein Otx2 is restricted to ventral tegmental area (VTA) neurons prevalently complementary to those expressing Girk2 and glycosylated active form of dopamine transporter (Dat). High level of glycosylated-Dat identifies neurons with efficient dopamine uptake and pronounced vulnerability to Parkinsonian degeneration. We found that Otx2 controls neuron subtype identity by antagonizing molecular and functional features of dorsal-lateral VTA such as Girk2 and Dat expression. Through this control, Otx2 limits the number of VTA neurons with efficient Dopamine uptake and confers resistance to the MPTP neurotoxin. Ectopic Otx2 expression also provides neurons of the substantia nigra with efficient neuroprotection to MPTP. These findings show that Otx2 is required to specify neuron subtype identity in VTA and may antagonize vulnerability to MPTP.

Phenotypic heterogeneity of Alzheimer's disease: towards the identification of molecular determinants underlying distinct clinico-pathological subgroups

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A remarkable aspect of the genetic forms of Alzheimer's disease (AD) is the considerable phenotypic heterogeneity which is due to variations of the cognitive profile and the occurrence of a broad spectrum of neurological deficits. Sporadic AD is commonly considered much less heterogeneous, presenting with a characteristic progressive amnestic disorder in most cases. However, other presentations, including behavioral, language or visual variants resulting in atypical clinical phenotypes, and occurrence of neurological abnormalities, such as extrapyramidal signs and myoclonus, are not infrequent. The molecular basis of phenotypic heterogeneity in AD is largely unknown. This is a critical aspect that has important diagnostic and therapeutic implications. To investigate this issue we carried out neuropathological studies on a series of patients with genetic and sporadic AD selected for having distinct clinical phenotypes, and found striking differences in brain regional distribution, topology, relative abundance and morphology of Aβ deposits. To unravel the molecular counterpart of these differences, we used an immunoproteomic assay enabling detection of the whole panel of Aβ using SELDI-TOF MS. Analysis of brain extracts and purified amyloid fractions from prototypic neuropathological AD subtypes showed remarkable differences in composition of Aβ species, resulting in specific fingerprints for each phenotype. Amyloidogenesis can be induced in mice by introduction of an exogenous seed (i.e., AD brain extracts). This process is governed by host and source of the Aβ-seed, reminiscent of prion stain propagation. On this ground we inoculated human APP overexpressing mice with brain homogenates from patients with different molecular subtypes of AD, and found that the phenotypic diversity of human pathology was partly maintained in the inoculated mice. These data suggest that the phenotypic heterogeneity is partly related to the biological properties of specific Aβ species.

Training course on "Mitocondria and Neurodegeneration: Novel Pathogenetic Mechanisms"

Friday, May 25th

The eye: a "mito-window" to the brain

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Since the early days of mitochondrial medicine, it has been clear that optic atrophy is a very common and, frequently, the only clinical feature in mitochondrial pathologies. In 1988, the first point mutation of mitochondrial DNA (mtDNA) affecting the respiratory complex I was associated with a maternally inherited blinding disorder, Leber's hereditary optic neuropathy (LHON). In 2000, another blinding disorder, dominant optic atrophy (DOA) Kjer-type, was found associated with mutations in the nuclear gene *OPA1* that encodes a mitochondrial protein implicated in mitochondrial dynamics and mtDNA maintenance. Besides the non-syndromic LHON and DOA and other primary mitochondrial encephalomyopathies, optic neuropathy is now recognized in many other neurodegenerative diseases including, for example, Parkinson's and Alzheimer's diseases. How retinal ganglion cells (RGCs) are selectively targeted in mitochondrial optic neuropathies remains the fundamental question to be answered. Anatomical structure and functional properties of the RGC neuronal system are investigated in patients and autopsy specimens, with special reference to myelination and axonal transport. The recent observation that a subset of RGCs, loaded with the photopigment melanopsin and functioning as photoreceptors, are largely spared by neurodegeneration in LHON and DOA opens new avenues to understanding pathogenic mechanisms. A major focus points now to mitochondrial biogenesis, dynamics and maintenance.

The role of mitochondria in the pathogenesis of Amiotrophic Lateral Sclerosis

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Recent studies suggest that the many, interconnected facets of mitochondrial dysfunction may play a more significant role in the etiopathogenesis of ALS than previously thought. This notion stems from accumulating evidence of the complex physiology of mitochondria and of alteration of their properties that might confer an intrinsic susceptibility to long-lived, post-mitotic motor neurons to energy deficit, calcium mishandling and oxidative stress. Such evidence has prompted new studies aimed to the development of new mitochondria-targeted therapies. We have recently demonstrated that two different genetic approaches allow rescue of correct mitochondrial function in models based on the expression of mutant SOD1 *in vitro* and *in vivo*. In particular, we have shown that overexpression of thiol disulfide oxidoreductases (Grxs) is an effective strategy to increase solubility of mutant SOD1 and that expression of mitochondrial Grx2 interferes with mitochondrial fragmentation and preserves mitochondrial function, strongly protecting neuronal cells from apoptosis. Moreover, deletion of 66 kDa isoform of the growth factor adapter Shc (p66Shc), known to be central in the control of mitochondria-dependent oxidative balance, ameliorates mitochondrial function, delays onset, improves motor performance and prolongs survival in transgenic mice modelling ALS. Our results provide new insight into the potential mechanisms of mutSOD1-mediated mitochondrial dysfunction and show that drugs targeting more than one aspect of mitochondrial dysfunction, or more than one molecular aspect of this devastating disease are needed for its successful treatment.

Peripheral neuropathy as dominant or unique presentation of mithocondrial disorders

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Peripheral nerves have peculiar energetic requirements because of length of axons and of myelin wrapping with several myelin lamellae layers, and correct mitochondria functioning is relevant for axonal transport. Many patients affected by different mitochondrial disorders (MD) have subclinical or mild peripheral neuropathy (PN). There are less common instances in which PN is the predominant or even the unique manifestation of a MD. Charcot-Marie-Tooth neuropathy Type 2A (CMT2A) associated with mutations in the gene encoding Mitofusin-2 (MFN2) is the main example. MFN2 is localized in the outer mitochondrial and endoplasmic reticulum (ER) membranes, thus regulating the mitochondrial fusion, and the ER-mitochondria juxtaposition. *MFN2* mutations may decrease mitochondrial fusion and cause sequestration of mitochondria to ER membranes, leading to mitochondrial trafficking abnormalities and mislocalization along the axons, thus resulting in energy starvation and axonal degeneration. Many different heterozygous *MFN2* mutations have been associated with CMT2A, an axonal autosomal dominant CMT type which in most cases is characterized by early onset, severe course and predominant motor involvement. In other instances, however, the disease runs a less severe course and has later onset. Optic atrophy (CMT5) and pyramidal involvement (CMT6) may occur. *De novo* mutations are particularly frequent. A few families with moderate to very severe axonal CMT have been recently found to harbor homozygous or compound heterozygous mutations in *MFN2*, showing a recessive or semidominant behavior with significant involvement only in patients carrying two mutated alleles. GDAP1 (ganglioside-induced differentiation-associated protein) is another CMT-associated protein which is localized in the outer mitochondrial membranes and may regulate mitochondrial dynamics. GDAP1 is expressed in neuronal cells more abundantly than in Schwann cells, and its mutations have been associated with both recessive, early-onset, severe CMT (CMT4A) and dominant axonal CMT2 (CMT2K). Relevant peripheral neuropathy may also occur in patients carrying mutations in OPA1, MAPT6 (NARP), POLG, PEO1 and TYMP (MNGIE).

Mitochondria, calcium and cell death

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Mitochondria rapidly accumulate Ca^{2+} through a low-affinity uptake system (the mitochondrial Ca^{2+} uniporter, MCU) because they are exposed to high [Ca^{2+}] microdomains generated by the opening of ER Ca^{2+} channels. These rapid [Ca^{2+}] changes stimulate Ca^{2+}-sensitive dehydrogenases of the mitochondrial matrix, and hence rapidly upregulate ATP production in stimulated cells. Ca^{2+} also sensitizes to cell death mediators, e.g., ceramide. Accordingly, we demonstrated that Bcl-2 reduces the state of filling of ER Ca^{2+} stores, and this alteration is effective in reducing the sensitivity to apoptotic challenges. I discuss two topics. The first is the current understanding of the link between calcium dysregulation in mitochondria and cell death, with special focus on neuromuscular diseases. The second topic is our recent discovery of the molecular identity of the MCU, i.e., the key molecule of mitochondrial Ca^{2+} homeostasis. I will present the strategy, and the experiments, that allowed to identify the protein, that remained elusive for 50 years. Then, I will present data that clarify the composition and the regulatory mechanisms of this highly sophisticated signaling machinery. Finally, I will that molecular targeting of MCU allowed novel insight into the regulation of cellular metabolism and cell death processes [1].

Reference

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Mitochondrial dysfunction in hereditary spastic paraplegias

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The *m*-AAA protease is an ATP-dependent metalloprotease embedded in the inner mitochondrial membrane, which performs quality control functions and regulates mitochondrial protein synthesis by mediating the maturation of the ribosome subunit MrpL32. The human *m*-AAA protease is a hexameric complex, formed by homo-oligomers of AFG3L2 or by hetero-oligomers of AFG3L2 and paraplegin. Heterozygous mutations in *AFG3L2* have been linked to dominant hereditary spinocerebellar ataxia SCA28, while loss-of-function of paraplegin (encoded by the *SPG7* gene) causes hereditary spastic paraplegia. Whether accumulation of misfolded polypeptides or impaired processing of specific substrates in mitochondria is the central pathogenic mechanism is still unclear. To address this question, we have developed animal models for deficiency of paraplegin or AFG3L2. Paraplegin-deficient mice show a late-onset progressive retrograde degeneration of long ascending and descending spinal axons, while *Afg3l2*$^{-/-}$ mice show a severe brain phenotype characterized by neuronal migration defects, impaired axonal and dendritic development, and reactive astrogliosis. To dissect early pathogenic events, we are employing a conditional *Afg3l2* mouse model to specifically delete the allele in Purkinje cells postnatally. Afg3l2$^{PC/PC}$ mice show a progressive loss of Purkinje cells associated with massive astrogliosis in the molecular layer, indicating that AFG3L2 is autonomously required for survival of these neurons. By identifying early alterations occurring before neuronal degeneration, we are reconstructing the pathogenic cascade.

Central nervous system (CNS) involvement in mitochondrial encephalomyopathies

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Cerebellar involvement, leukoencephalopathy, bilateral striatal necrosis are all manifestations of specific mitochondrial disorders. Subclinical CNS involvement is also present in the apparently pure myopathic PEO. Epilepsy is relatively common, particularly in MERRF and MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) syndromes. MELAS is characterized by sudden neurological deficits similar to vascular strokes, but the pathophysiology of stroke-like episodes (SLEs) is still debated. Out of 41 SLEs in 9 of our patients with A3243G mutation 64% followed migraine and 74% were associated with seizures. Attacks involved occipital (62%), parietal (59%), and temporal (51%) lobes. Acute lesions were bilateral in 66% of SLEs and symmetric at onset in 51% with a very high incidence, compared with vascular strokes, of bilateral clinical syndromes as cortical visual loss or cortical deafness. Brain MRI showed large T2-bright lesions in supratentorial cortical and subcortical areas with non-vascular distribution and migrational behavior. Lesions were DWI-bright, with mixed pattern of restricted and increased diffusion on ADC maps. Follow-up MRIs demonstrated partial reversibility of the lesions, with subsequent development of pseudolaminar necrosis and gliosis. However cerebral and/or cerebellar atrophy by far exceeded the extension of SLEs demonstrating two mechanisms playing in MELAS patophysiology: abrupt loss of function due to cell injury followed by partial recovery and an independent slowly progressive degenerative process. Beside classical CNS manifestations, dementia and extrapyramidal signs may be also present in mitochondrial disorders sometime dominating the phenotype. Neuroimaging, MRI spectroscopy and functional study may help to better characterize these features versus non mitochondrial neurodegenerative diseases.

Mitochondrial maintenance and spinocerebellar degeneration

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Hereditary ataxias are a complex and heterogeneous group of neurodegenerative disorders primarily affecting the cerebellum and/or its afferent and efferent connections. More than 30 distinct genetic forms are currently known, with great heterogeneity of the molecular mechanisms causing the neurodegeneration. Mutations in nuclear genes encoding mitochondrial proteins can be observed in a growing number of cases. However, none of these genetic defects directly involve primary players of the mitochondrial energy production system, such as the respiratory chain complexes or other enzymes of the oxidative cycles, whose deficiency commonly manifest with generalized dysfunction of tissue and organs sensitive to aerobic energy production. The spinocerebellar systems, which include some of the longest paths of the human nervous system, appear to be particularly vulnerable to genetically-determined abnormalities of proteins involved in the maintenance of mitochondrial integrity and function. Frataxin, which causes Friedreich ataxia, the most common form of hereditary ataxia, is involved in the byosynthesis of Fe-S clusters which constitute the essential reactive centers of many mitochondrial and extramitochondrial proteins. DNA polymerase gamma and the helicase twinkle cause recessive forms of ataxia and are involved in mitochondrial DNA replication. More recently, the crucial role of protein quality surveillance and integrity of the mitochondrial proteasome in protecting the spinocerebellar system against neurodegeneration has been highlighted by our finding that mutations in AFG3L2, a partner of paraplegin in the inner membrane *m*-AAA metalloprotease, cause a dominantly-inherited form of spinocerebellar ataxia (SCA28) [1]. (Supported by Telethon grant GGP09301).

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Pathways of mitochondrial dysfunction in Parkinson's disease

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Parkinson's disease (PD) is a common neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and by the accumulation, within surviving neurons, of typical cytoplasmic protein inclusions (the Lewy bodies). While the majority of cases are sporadic, in a subset of patients the disease is caused by pathogenic mutations in genes with either autosomal dominant (SNCA/α-synuclein and LRRK2/dardarin) or recessive (Parkin, PINK1 and DJ-1) inheritance. Research on these genes, as well as epidemiological studies in humans and toxin-induced animal models of PD, helped to identify mitochondrial dysfunction and failure of the protein degradation machinery as major determinants of PD etiology. In particular, the activity of the respiratory Complex I is significantly reduced in the SNc of PD patients, possibly due to increased oxidative stress generated by dopamine metabolism. Moreover, mutations in nuclear-encoded mitochondrial proteins and mtDNA deletions can contribute to PD pathogenesis in some instances. Besides compromising energy production, dysfunctional mitochondria can also mediate the induction of apoptosis, especially upon cellular stress. In addition, the dynamic properties of mitochondria (fission/fusion, biogenesis and trafficking) have known to play a major role in regulating neurotransmission, synaptic plasticity and neuronal survival, and a defect in mitochondria selective removal through autophagy (termed "mitophagy"), has recently emerged as a key mechanism

of disease. Collectively, mitochondrial dysfunction represent a key step in PD pathogenesis; drugs and genetic approaches aimed at modulating mitochondrial dynamics, homeostasis and function are expected to have important clinical applications in future treatments of PD.

Workshop 3 "Biomarkers and Neuropathologic Processes"

Friday, May 25th

Biomarkers for neurometabolic hereditary diseases

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Neurometabolic hereditary diseases comprise a group of inherited metabolic disorders which affect the nervous system more than other organ system. Therefore, patients show neurological signs and symptoms associated with other organ symptoms. Inherited metabolic disorders are the result of a disturbance in the metabolism of proteins, lipids, carbohydrates or nucleic acid. In most of the disorders the subsequent accumulation of a toxic substance can damage the cell or the subcellular components (e.g., mitochondria or lysosomes). The same disease may have different presentations, depending on the age of onset, with "attenuated" phenotypes in adulthood. Moreover, there is a frequent overlap in many clinical manifestations and the clinical diagnosis can be very difficult. For this reasons, a specific diagnosis cannot be achieved only on clinical basis alone. The National Institutes of Health defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". For neurometabolic inherited diseases, biomarkers are needed to aid diagnosis, monitor disease progression and detect, when possible, response to treatment. The presentation will include examples of the use of biomarkers in some neurometabolic hereditary diseases: oxysterols as blood-based biomarkers for Niemann-Pick Type C, a lysosomal neurodegenerative disease; CSF neurotransmitters for the investigation of the defects of the biogenic monoamines; N-acetylaspartate (NAA) as a biomarker for the diagnosis of hyperacetylaspartia and hypoacetylaspartia. Furthermore, the NAA-NAAG system will be discussed as biomarker of bioenergetic changes in human brain condition.

Neuroproteomics for studying neural differentiation and function

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Recent developments in proteomic technologies applied to neuroscience ("neuroproteomic") offer new opportunities for understanding the molecular networks regulating neuronal differentiation and function [1]. Neuroproteomic studies on CNS disorders constitute a dynamic research area in biomedicine and are increasingly employed in neurological research to provide insight into the molecular basis of neurological diseases. In this framework, the large-scale characterization of embryonic and neural stem cells has been performed by applying an innovative label-free qualitative and quantitative LC-MS analysis [2, 3]. In addition, the secretome profile of murine embryonic stem cell following cardiac and neural differentiation has been also performed allowing the identification of potential mediators of differentiation processes [4]. This new methodology, for the first time applied to the investigation of stem cell differentiation, proved to be very powerful for the profiling of changes occurring upon neural differentiation, thus opening new frontiers for the study of signal transduction pathways involved in stem cell commitment.

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Biomarkers for Alzheimer's disease and related dementias

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In the last few years, new criteria for Alzheimer's disease (AD) and the behavioral variant frontotemporal dementia (bvFTD), that are the most frequent neurodegenerative diseases with, respectively, a senile or presenile onset (< 65 years), have been proposed. Both of them are aimed to anticipate the diagnosis and include biomarkers in the clinical workup. Regarding AD, there are four possible biomarkers: 1) structural imaging (MRI) 2) functional imaging (PET-SPECT) 3) cerebrospinal fluid (CSF) biomarkers amyloid β, τ and phosphorylated τ 4) presence of an autosomal dominant causal mutation. According to these criteria, in the presence of a progressive cognitive decline and positivity for one of the above biomarkers, the diagnosis of prodromal AD can be done. These criteria anticipate the time of diagnosis, i.e., even when the clinical symptoms are not overt although biomarkers suggest an ongoing pathology. The level of certainty is met in the presence of a causal mutation; in this case the term "presymptomatic AD" is advised in the absence of symptoms. Concerning bvFTD, new criteria classify the disease in "possible", "probable" or "definite" basing

on the presence of clinical symptoms only, or symptoms plus positivity for a radiological marker, or the presence of a causal mutation. In bvFTD workup, CSF analysis is useful for excluding AD, but there are no specific bvFTD alterations.

Markers of autoimmunity in autoimmune encephalitis

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Encephalitis is classically defined as an inflammatory process of the brain caused by a viral or bacterial infection. However, several pictures of encephalitis that are clinically and pathologically indistinguishable from the infectious forms, are known to be associated with autoimmune mechanisms. In recent years the field of autoimmune mechanisms has undergone extensive development. The study of paraneoplastic forms of encephalitis, in particular, has led to the identification of new antibodies associated with specific clinical pictures, some of which are non paraneoplastic. In these diseases, cell-mediated and humeral immunity have an important role in bringing about an inflammatory response in the CNS, in the presence or absence of an extracerebral tumor. Interest in these diseases has been increasingly growing, partly as a result of the description of several very common clinical phenotypes in the neurological domain, such as temporal epilepsy and dementia. This importance is all the more salient considering that these forms of autoimmune encephalitis are potentially responsive to immunomodulating or immunosuppressive treatments. The main intracellular and surface-membrane autoantibodies against neuronal antigens that play an important role in the diagnosis of autoimmune forms of encephalitis, will be presented and discussed.

Workshop 4 "From Pathogenesis toward Therapy"

Saturday, May 26th

Multifactional delivery scaffolds for functional skeletal muscle regeneration

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The main strategies currently pursued for tissue regeneration are typically limited by the death of the majority of the transplanted cells. Most of the drug delivery strategies have yielded limited success, likely related to rapidly depleted local concentrations, inappropriate gradients, and/or loss of bioactivity of growth factors (GFs) via bolus delivery and rapid degradation in the inflammatory in vivo environment. Biodegradable polymeric systems have been developed to provide localized and sustained GF release. In addition, regenerative efforts typically focus on the delivery of single factors or cells, but it is likely that multiple factors regulating distinct aspects of the regenerative process will need to be used in parallel to affect regeneration of functional tissues. We have been addressing this concept using alginate-based vehicles. The combined delivery of VEGF and IGF1 via alginate-based gels in ischemic rodent hindlimbs [1] led to parallel angiogenesis, reinnervation, and myogenesis; as satellite cell activation and proliferation was stimulated, cells were protected from apoptosis, the inflammatory response was muted, and highly functional muscle tissue was formed. Based on these findings we then investigated whether alginate-based polymeric scaffolds could direct culture-expanded myoblasts to migrate outwards and repopulate the host damaged tissue, in concert with release of VEGF and IGF to enhance the efficacy of this cell therapy and promote functional muscle regeneration. This was investigated in the context of a severe injury to skeletal muscle involving both myotoxin-mediated direct damage and induction of regional ischemia. Local and sustained release of VEGF and IGF-1 from macroporous scaffolds used to transplant and disperse cells significantly enhanced their engraftment, limited fibrosis, and accelerated the regenerative process. This resulted in increased muscle mass and improved contractile function [1]. These results demonstrate an improved transplant strategy that enhances the ability of cultured myoblasts to engraft and promote functional regeneration. These material platforms provide a significant advance by supplying an adequate environment to direct cell migration and differentiation, and lead to tissue healing and maturation. This approach to tissue regeneration may be useful in a variety of clinical situations beyond muscle injury, including bone and cardiovascular tissue regeneration.

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Alternative sources of dopaminergic neurons for cell replacement therapy in Parkinson's disease

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Degeneration of mDA neurons is associated with Parkinson's disease (PD), one of the most common human neurological disorders. Previous studies using fetal grafts in PD patients have indicated that cell replacement therapy can result in significant symptomatic relief. However, this treatment is often associated with the development of serious side-effects eventually caused by the concomitant transplantation of non-DA neurons. Therefore, it has been suggested that a highly selective source of DA neurons would provide a better clinical outcome. In fact, embryonic stem (ES) cell-derived DA neurons have shown to be efficient in restoring motor symptoms when transplanted in PD animal models. However, the use of pluripotent derived cells might lead to the generation of tumors if not proper-

ly controlled. Thus, the development of alternative approaches to generate DA neurons independently by stem cells might represent an interesting opportunity. Lineage-specific transcription factors, which drive cellular identity during embryogenesis, have been shown to convert cell fate when expressed ectopically in heterologous cells, without passing through a proliferating step. Herein, we screened the key molecular factors governing the dopaminergic (DA) neuronal specification during brain development for their ability to generate functional neurons directly from mouse and human fibroblasts. Remarkably, we found a minimal set of three factors *Ascl1*, *Nurr1* and *Lmx1a* able to elicit such cellular reprogramming. Molecular and transcriptome studies showed that reprogrammed DA neuronal cells recapitulate gene expression of their brain homolog cells. Induced DA neuronal (iDAN) cells released DA and showed spontaneous electrical activity organized in regular spikes consistent with the pacemaker activity featured by brain DA neurons [1]. Generation of iDAN cells from somatic cells might have significant implications in studies of neural development, disease *in vitro* modeling and cell replacement therapies.

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New multi-target-directed ligands as potential therapeutic agents for Alzheimer's disease

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Alzheimer's disease (AD) is the most common cause of dementia, clinically characterized by loss of memory and progressive deficits in different cognitive domains. An emerging disease-modifying approach to face the multifactorial nature of AD may be represented by the development of multi-target-directed ligands (MTDLs), i.e., single compounds which may simultaneously modulate different targets involved in the neurodegenerative AD cascade [1]. In parallel, since a significant number of therapeutic targets of AD reside inside cells and intracellular organelles, subcellular targeting strategies for drug design and delivery now represent a considerable drug discovery challenge [2]. In the search of new rationally designed MTDLs against AD, we synthesized cystamine-tacrine dimer, based on structure of the acetylcholinesterase (AChE) inhibitor tacrine [3]. "In vitro" assays were performed on the new dimer to determine inhibition of human AChE and butyrylcholinesterase (BChE) and beta-amyloid (Aβ) aggregation, neuronal viability of neuroblastoma SH-SY5Y cell line, effect on intracellular ROS formation, and the role of ERK1/2 and Akt pathways in neuroprotection. Results: In this study we demonstrated that the cystamine-tacrine dimer is endowed with a low toxicity, it is able to inhibit AChE, BChE, AChE-induced Aβ aggregation and exerts a neuroprotective action on SH-SY5Y cell line against H_2O_2-induced oxidative injury, by activating Kinase 1 and 2 (ERK1/2) and Akt/protein kinase B (PKB) pathways. Cystamine-tacrine dimer emerged as a new MTDL which may be potentially useful in AD treatment, thanks to its well balanced biological profile as cholinesterases inhibitor and cytoprotective agent.

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A vaccine for glioma: the role of dendritic cells

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Glioblastoma is the most common primary brain tumor in humans with the most severe prognosis. Current therapeutic approaches give to brain cancer patients limited benefits and quite short survival from diagnosis. Accumulating evidences indicate that immunotherapy or "vaccines" may prove effective for the treatment of cancer. Numerous reports have shown tumor regression, proving that the immune system can be harnessed to kill tumor cells in cancer patients. The absence of severe side effects associated with this therapeutic strategy make these treatments particularly promising to be also used in combination therapy. The possible goal of vaccination relies today in the long term control of cancer disease or at least to prolong life with optimal quality and minimal side effects. Different strategies can be adopted to trigger immune response against tumors. Dendritic cells (DC) represent the most promising approach for cancer vaccination. Clinical trials have demonstrated that DC-based vaccination is feasible, well tolerated and possibly beneficial for patients with minimal residual tumor burden. DCs are the key regulators of immune system due to their capacity to activate B and T cells. These cells are able to phagocyte, process and present antigens at very high efficiency. The possibility to obtain in vitro a large numbers of DCs together with the know-how that allows manipulation and control of cell differentiation has stimulated the experimental research to translate this to the clinic as cell therapy. Further characterizations are however still necessary to plan optimal vaccination schedule and to fit this new strategies with conventional protocols of treatment like surgery and chemotherapy.

Estrogen-dependent molecular mechanism(s) of neuronal survival

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Estrogens (E_2) promote a plethora of effects in the CNS that profoundly affect both its development and mature functions and are able to influence proliferation, differentiation, survival and neurotransmission. The biological effects of E_2 not only are cell-context specific but in a given cell type also depend on differentiation and/or proliferation status. E_2 activate ERK1/2 (ERKs)s in a variety of cellular types. We investigate whether ERKs activation might be influenced by E_2 stimulation according to the differentiation status and eventually the molecular mechanisms underling this phenomenon. ERKs exert an opposing role on survival and death, or on proliferation and differentiation depending on different kinetics of phosphorylation [1]. We report that mesencephalic primary cultures and an immortalized cell line, mes-c-myc A1 (A1), express estrogen receptor α (ER-α) and activate ERKs upon E_2 stimulation. Interestingly, following the arrest of proliferation and the onset of differentiation, we observe a change in the kinetic of ERKs phosphorylation induced by E_2 stimulation. Moreover, caveolin-1 (cav-1), a main constituent of caveolae endogenously expressed and co-localized with ER-α on plasma membrane, is consistently up-regulated following differentiation and cell growth arrest. In addition, we demonstrated that siRNA-induced cav-1 down-regulation or disruption by means of β-cyclodextrin treatment, changes ERKs phosphorylation in response to E_2 stimulation. Finally, cav-1 down-regulation abolishes E_2-dependent survival of neurons. Thus, cav-1 can be important player in mediating, at least, some of the non-genomic action of E_2 in neurons, in particular ERKs kinetics of activation and survival.

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Oral and Poster Communications

May 24th – 26th

Role of SIRT3 in human longevity

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Introduction: Sirtuins (SIRT) are proteins involved in lifespan modulation, caloric restriction and basic cellular homeostasis. SIRT3 is involved in oxidative stress response and longevity in animal models and human studies. Materials and methods: To investigate the involvement of SIRT3 in longevity, we collected blood from around 1200 elderly people aged between 70 and 110 years (TRELONG Study and Monzino80+ Study, two prospective studies recruiting people from North Italy), than we performed DNA extraction. Allelic discrimination RT-PCR was done to evaluate subjects' genotype using TaqMan probes . The SIRT3-SNP (rs11555236) was selected on the basis of literature data. Results: We found evidence of association of the minor T-allele to longevity after plotting a survival curve ($p = 0.036$ of the TT genotype in comparison to GG). The molecular mechanisms underlying this positive effect might deal with antioxidant function of SIRT3. Conclusions: Our analysis demonstrates a correlation between SIRT3 rs11555236 and longevity.

DNA repair and cell death after antitumor drugs in glioblastoma cell lines

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Introduction: Resistance to radio- and chemotherapy is a recognized feature of cancer stem cells (CSC), beside their expression of stemness antigens and genetic alterations similar to those of primary tumors. Our goal is to study the cascade of DNA repair and cell death/apoptosis in glioblastoma (GBM) cell lines after chemotherapeutics. Material and methods: Cell lines were obtained from GBM samples put in culture with DMEM/F12 with growth factors or serum. Neurospheres (NS) and adherent cells (AC) developed, respectively. Temozolomide and doxorubicin were administered with different action times and concentrations. The cascade of DNA repair and apoptosis or necrosis were studied with immunohistochemistry and immunofluorescence by a series of relevant antibodies: gH2AX, p-ATM, 53BP1, p-Chk2, Ku70/80, DNA-PK, RAD51, kit for apoptosis, Comet assay and cell counts. Autophagy was evaluated as well. Results: Preliminary results can be summarized as follows. High concentrations and long action times of drugs are needed in order to obtain modifications of cell responses. Comet assay positivity is in relation with drug action times and concentrations. The first responses are those of gH2AX, p-ATM, p-Chk2 and DNA-PK, whereas apoptosis appears later. The number of living cells decreases with drug action times and concentrations. This phenomenon is much more evident in AC than in NS. Conclusions: The need of high drug times and concentrations to elicit cell responses and death is in line with what is known from the clinical use of temozolomide. The earlier appearance of some cell responses in comparison with apoptosis, necrosis and autophagy means that what needs more time is the intermediate mechanism between the sensor activation and the effective cell damage or repair. The non-homologous pathway of DNA repair

(NHEJ) seems more active than the homologous one (HR). Autophagy may occur in spite of the functioning of the efflux pump.

BRAF-KIAA1549 fusion gene and IDH mutations are independent genetic events in adult gliomas

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BRAF-KIAA1549 fusion gene and *IDH* mutation have been considered as two genetic events, occuring in a mutually exclusive manner in pilocytic astrocytomas (PAs) and diffuse gliomas, respectively. After discovering cases of adult patients with oligodendroglioma displaying *BRAF-KIAA1549*, we investigated the presence of this alteration in conjunction with *IDH* mutation and 1p/19q loss in 185 diffuse gliomas and 31 non-diffuse gliomas in patients aged > 20 years. The *BRAF-KIAA1549* fusion gene was evaluated by RT-PCR and sequencing; *IDH1* and *IDH2* mutations by direct sequencing; and 1p/19q loss by microsatellite analysis or CGH. In diffuse gliomas, we found *IDH* mutations in 118 out 175 cases (67%) and the *BRAF-KIAA1549* fusion gene in 17 cases (9%). Among these 17 cases, oligodendrogliomas were more common (6.5%). In 11 of these 17 cases, both *IDH* mutation and the *BRAF-KIAA1549* fusion were present, indicating that these molecular alterations are not mutually exclusive events. Moreover, 6 of 17 cases showed co-presence of 1p/19q loss, *IDH* mutations and *BRAF-KIAA1549* fusion. Our results suggest that in a small fraction of diffuse gliomas the *KIAA1549-BRAF* fusion gene may be responsible for deregulation of the Ras-RAF-ERK signaling pathway. In addition, such alteration may be co-present with *IDH* mutations and 1p/19q loss.

Doxycycline reverts oligomer-mediated memory impairment in mouse models of Alzheimer's disease

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Introduction: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by deposition of extracellular beta-amyloid (Aβ) plaques and intracellular neurofibrillary tangles. The inability to form new memories is an early clinical sign of AD and soluble Aβ oligomers are proposed as key mediators of synaptic and cognitive dysfunction. Here we validated a new, reliable and simple acute mouse model in which synthetic $A\beta_{1-42}$ oligomers impair recognition memory, providing a new tool to test the efficacy of new therapeutic approaches directly against oligomer action. Since tetracyclines do interfere with Aβ aggregation, we tested the effect of the antibiotic doxycycline (doxy) in aged APP/PS1 transgenic (Tg) mice and directly against oligomers in the acute model. Methods: Tg mice were intraperitoneally (IP) treated both acutely and sub-chronically with doxy and subsequently evaluated behaviorally in the object recognition test (OR) and histologically for Aβ plaque deposition. In the acute model, intracerebroventricle injection of Aβ oligomers (1.0 mM) were preceded by doxy treatment, at specific intervals pre-Aβ oligomer infusion, and then tested in the OR. Results: Our findings show that doxy significantly antagonizes memory deficit in Tg mice without affecting plaque deposition suggesting an action on Aβ oligomers. This was demonstrated by the significant effect of the drug to antagonize the oligomer-mediated memory impairment in the acute mouse model. Conclusions: Our data promotes doxy as valuable therapeutic approach against Aβ oligomers, showing a protective effect also in a more compromised system such as that of aged APP/PS1Tg mice with an important burden of amyloid plaques.

Genetic testing in frontotemporal lobar degeneration: the role of C9ORF72 repeat expansion in Northern Italy

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Introduction: Frontotemporal lobar degeneration (FTLD) is highly heritable: up to half of patients have a family history of the disease and genetic factors are the only cause of FTLD so far identified. Expanded GGGGCC hexanucleotide repeat in C9ORF72 was recently implicated in FTLD. Herein we examined the role of this novel genetic determinant in FTLD patients form Northern Italy. Materials and methods: A total of 297 FTLD patients (n = 117 with positive family history) were enrolled at the Memory Clinic, IRCCS Istituto Centro San Giovanni di Dio Fatebenefratelli, Brescia and IRCCS Istituto Neurologico Carlo Besta, Milan. The qualitative assessment of the expanded hexanucleotide repeat in C9ORF72 was performed by repeat primed PCR reaction followed by fragment analysis, after pre-screening with PCR amplification and gel analysis of the expanded region. Results: The C9ORF72 repeat expansion was detected in 15 unrelated familial FTLD patients (12.8%) and 4 sporadic FTLD cases (2.2%), leading to a prevalence of 6.4% within the whole cohort. The majority of patients were diagnosed with behavioral variant Frontotemporal Dementia, 4 with FTD-MND. Age at onset ranges from 41 to 62 years. Segregation of C9ORF72 expansion with the disease was demonstrated in 4 large pedigrees with an autosomal dominant pattern of inheritance. Conclusions: The expanded hexanucleotide repeat in the C9ORF72 gene is a common genetic determinant in Northern Italy, especially in patients with a positive family history. The analysis of this gene should be could be incorporated into the recently developed algorithm of genetic testing for FTLD.

Aging lesions in bovine muscles: a comparative study with human sarcopenia

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Introduction: Aetio-pathogenesis of sarcopenia is complex and probably involves several hormonal, metabolic and nutritional factors as well as physical inactivity. The aim of this work was to study the age related lesions in the skeletal muscles of cattle. Materials and methods: Muscle samples (diaphragm and sternomastoid muscle) from 34 aged cows (7 – 20 years) and 5 calves (18 – 24 months) as controls, were submitted to histological, histochemical and immunohistochemical staining to evaluate morphology, oxidative activity and inflammatory reaction. Thiobarbituric acid reactive sustances (TBARS) were determined in order to assess the extent of lipid oxidation. Results: Internal nuclei, angular fibers, fibers atrophy, necrosis, focal sarcosporides, non suppurative inflammatory infiltrates (positive for CD4, CD8, CD79, and MHC1) and increase of connective tissue were the most important detected features. Older animals (16 – 20 years) showed a significant higher number of internal nuclei (ANOVA, $p < 0.01$). A significant decrease of TBARS products in the diaphragm was detected in the same group of cattle compared with the calves (Wilcoxon-Mann Whitney test, $p < 0.05$). Discussion and conclusions: Degenerative and regenerative changes or denervation injuries suggest similarities between sarcopenia in humans and cattle. Preliminary data of TBARS analysis suggest that histological featured detected are not related to the oxidate damage. Further investigations are needed to better understand the mechanism of these muscular changes.

Loss of progranulin (PGRN) and exosomal pathogenetic proteins trafficking

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Introduction: Exosomes are small vesicles secreted by most cell types after the fusion between the multivesicular bodies (MVB) and the plasma membrane. They play important roles in intercellular communications. Also neurons and glial cells can secrete proteins, lipids, and genetic material by exosome pathway. It has recently been discovered that proteins associated with neurodegenerative disorders can be released within exosomes [1, 2]. A better understanding of the exosomal mechanism may be important for the study of neurodegenerative diseases such as frontotemporal lobar degeneration (FTLD), caused by progranulin (PRGN) loss. Materials and methods: Exosomes are isolated from human SH-SY5Y cell medium. The 24 hours conditioned medium was centrifuged at different speed with a last ultracentrifugation at 100,000 g for 70 min. Exosomes were then characterized by Western blot. Results: On account of the strong phenotipic variability of FTLD, we decided to study if PGRN loss may alter the exosomal sorting of other neurodegenerative disorders proteins such as, α-synuclein, β-APP, TDP43, τ and DJ1. In order to evaluate if loss of PGRN can determine the accumulation of that proteins in exosomes we knock down PGRN by using specific siRNA. In non-interferenced SH-SY5Y cell we have noticed that α-synuclein is secreted by exosomes while PGRN and DJ1are not. Conclusions: In neurodegenerative disorders the mechanism underlying cell death could involve transport of toxic agents or accumulation of pathogenetic proteins by exosomes [3]. In this context, the loss of PGRN could modify proteins trafficking, affecting the fate of neurons.

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CYP2D6 polymorphism rs1080985 as pharmacogenetic marker in Alzheimer's disease patients treated with donepezil

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Introduction: Alzheimer's disease (AD) is treated by acetylcholinesterase inhibitors (AChEI), including donepezil. Individual responsiveness to donepezil administration is variable, also on account of genetic polymorphisms. We have investigated candidate polymorphisms located in CYP2D6 and APOE genes. CYP2D6 codes for a cytochrome involved in donepezil metabolism, while the presence of the APOE-ε4 allele might influence the outcome of the therapy. Materials and methods: Genomic DNA was extracted from blood sample of 415 Italian AD subjects treated with donepezil and divided into responders and non responders according to cognitive performance assessment over 6 months (measured by MMSE variation). We genotyped polymorphisms in CYP2D6 and APOE genes by allelic-discrimination real-time methodology. Results: We have genotyped the CYP2D6 single nucleotide polymorphism *rs1080985,* that was associated to non responders (OR [95%CI]: 1.74 [1.01 – 3.00], $p = 0.04$) after multivariate analysis taking into account age, sex, and ApoEe4 status. We found no association as for ApoE-ε4 alone. Conclusions: We were able to confirm CYP2D6 rs1080985 as short-term marker for donepezil response even though the variance explained is limited.

Relevance of a panel of cerebrospinal fluid biomarkers in patients with a clinically isolated syndrome suggestive of Multiple Sclerosis

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Introduction: Clinically isolated syndrome (CIS) is the most frequent type of onset in Multiple Sclerosis (MS). However, not all CIS cases necessarily convert to MS and predicting the evolution of CIS remains a challenge. Cerebrospinal fluid biomarkers (CSF), such as 14-3-3 proteins, τ-protein, and possibly cystatin C, are promising source of information in this area. Materials and methods: Immunoblot analysis and Sandwich enzyme-linked immunoassorbent assay (ELISA) were carried out on CSF sample from 30 subjects with CIS, 6 neuromyelitis optica (NMO), 6 idiopathic transverse myelitis (ATM), 11 non-inflammatory/non-neurodegenerative disorders (NINDDs) (negative control), and 8 with sporadic Creutzfeldt-Jakob disease (sCJD) (positive control), to detect 14-3-3 proteins, τ-protein and cystatin C. Results: CSF of CIS and NMO/iATM patients resulted 14-3-3 positive or weak positive more frequently than NINDDs, while 14-3-3 was positive in all sCJD cases. NMO/iAMT patients showed significantly higher τ levels compared to both NINDDs and CIS, even if lower compare to sCJD cases. Mean level of cystatin C was comparable in CIS, NMO/iAMT and NINDDs cases, albeit significantly higher in sCJD compared to the other group, with the exception of NMO/iAMT cases. To provide additional insights on cystatin C conformational state we determined CSF levels by both Western Blot and ELISA. Conclusions: 14-3-3 and τ-protein were up-regulated in the CSF of CIS patients included in this study and may be helpful in monitoring pathological processes in demyelinating diseases. CSF τ showed potential diagnostic utility in differentiating between CIS and NMO/iAMT. Further research is needed to better clarify the possible diagnostic and prognostic relevance of cystatin C.

TMEM106B genetic variability in patients with Alzheimer's disease

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Introduction: Recently, the uncharacterized transmembrane protein 106B gene (*TMEM106B)* on chromosome 7p21.3, has been identified as a novel risk factor for frontotemporal lobar degeneration (FTLD) with TDP pathology, acting by modulating the levels of secreted progranulin. The main aim of the study has been to test whether *TMEM106B* genetic variability is associated with AD and to determine its possible influence on plasma progranulin levels. Methods: An association analysis of *TMEM106B* single nucleotide polymorphisms (SNPs) rs1020004, rs6966915 and rs1990622, covering the whole genetic variability of the gene, was carried out in a population of 300 patients with Alzheimer's disease (AD) compared with 323 age-matched controls. In addition, plasma progranulin levels were analyzed in 80 AD patients. Results: Considering *TMEM106B* variants, no differences were found both in allelic and genotypic frequencies in patients compared with controls. Stratifying according to age at onset or gender no differences were found as well. No differences in progranulin plasma levels were found after stratification according to rs1990622 status (A carriers: 130 ± 3.2 ng/ml vs. G carriers: 135 ± 4.1 ng/ml). Conclusions: according to these preliminary results, *TMEM106B* does not appear to act as susceptibility factor for AD. Moreover, rs1990622 does not seem to exert an influence on circulating progranulin levels, in contrast with data previously described in patients with FTLD.

Dopamine induces apoptosis in human APP-expressing Neuro2A cells following proteolysis of APP in endosomal compartments

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Introduction: Alzheimer's disease (AD) often associates with parkinsonian symptoms, which is suggestive of dopaminergic neurodegeneration. A pathological hallmark of AD is the presence within neurons and the interneuronal space of aggregates of β-amyloid (Aβ) peptides, that originate from an abnormal proteolytic processing of the amyloid precursor protein (APP). The aspartyl proteases that initiate this processing act in the Golgi and endosomal compartments. Aim and methods: The effects of dopamine, a neurotransmitter diffused in substantia nigra, Striatum and other brainstem nuclei, on the trafficking and processing of APP and neuronal cell survival were studied in neuroblastoma Neuro2A cells over-expressing human APP695. Results: Dopamine induces endosomal translocation of APP in Neuro2A neuroblastoma cells and promotes amyloidogenic processing of APP within endosomes. Amyloidogenic processing of APP eventually causes lysosome leakage and apoptosis. Pepstatin A and chloroquine inhibit dopamine-induced processing of APP and prevent apoptosis induced by dopamine. Conclusions: Our data shed lights on the mechanistic link between AD and Parkinson's disease, and provide the rationale for the therapeutic use of lysosomal activity inhibitors such as chloroquine or Pepstatin-A to alleviate the progression of AD leading to onset of parkinsonism. (Research supported by grants from San Paolo (Project Neuroscienze 2008.2395), Regione Piemonte (Ricerca Sanitaria Finalizzata, Torino), Comoli, Ferrari & SpA (Novara, Italy)).

The possible correlation between blood lactate during ischemic exercise test, pathological and biochemical muscle findings in patients with suspected mitochondrial myopathy

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Introduction: In mitochondrial myopathies the impairment of oxidative phosphorylation, with enhanced lactate production, can be elucidated by a simple, non-invasive screening tool that is the aerobic forearm test. However, this test, commonly used in the clinical practice in our Unit, is neither sufficiently specific nor sensitive for the diagnosis of mitochondrial disorders. The idea that a positive aerobic forearm test (increased lactate values after exercise) reflects an altered oxidative phosphorylation remains speculative. Methods: In order to better understand this issue, we retrospectively evaluated if there was an association between the results of the ischemic test and the results of the biochemical analysis of the respiratory chain in muscle specimens in a group of 59 unselected patients (26 females, 33 males; age 46.3 ± 14.4 years) who underwent both examinations during their diagnostic work-up for suspected mitochondrial disease (years 2008 – 2010). We also correlated these data with the muscle morphological abnormalities consistent with a diagnosis of mitochondrial myopathies observed with Gomori's Trichrome, ATPase and oxidative staining (NADH, SDH, COX, double-staining for COX and SDH). Results: We observed a significant association between increased ischemic lactate and reduced NADH:ferricyanide-reductase (Complex I) activity. Interestingly, no patient with normal ischemic test had reduced NADH:ferricyanide-reductase activity. Conclusions: The purpose of this study was to understand if the ischemic test may somehow predict the biochemical results, much before the definitive etiopathogenetic characterization of the patient. To our knowledge, previous studies showing similar results are not available. NADH:ferricyanide reductase activity is commonly used as a biochemical marker of Complex I function; it is the first component of complex, thus forming the major entry-point of electrons into the oxidative phosphorylation. For this last reason, dysfunction of this enzymatic activity may potentially act as a metabolic block in aerobic metabolism, with enhanced lactate production.

Cerebrospinal fluid findings in patients with chronic inflammatory demyelinating polyradiculoneuropathies (CIDP) with and without diabetes

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Introduction: CIDP is a chronic acquired neuropathy which occur more commonly in patients with diabetes mellitus. Differential diagnosis between diabetic neuropathy and CIDP in diabetes is traditionally based only on neurophysiological criteria. Objective: To evaluate features of cerebrospinal fluid (CSF) in idiopathic CIDP (I-CIDP) vs. CIDP with diabetes mellitus (DM-CIDP). Methods: We studied CSF of 52 patients diagnosed with CIDP from 2005 to 2011. 35 patients were affected by I-CIDP and 17 by DM-CIDP. Results: We found elevated CSF protein content in 36 patients (69%) affected by CIDP; 21/35 (60%) were subjects with I-CIDP and 15/17 (88%) subjects with DM-CIDP ($p = 0.055$). Among these, protein content was > 1 g/l in 22.8% of I-CIDP and 35% of DM-CIDP ($p = 0.050$). There was increased permeability of the blood brain barrier in 40/51 CIDP, 88% in DM-CIDP and 73% in I-CIDP. Oligoclonal bands were found in 33% I-CIDP and 70% DM-CIDP ($p = 0.016$). Only one case of IgG intrathecal synthesis was found in I-CIDP. Conclusions: This study confirms that elevated CSF proteins is a supportive criteria not always fulfilled in the diagnosis of CIDP. However, CSF study seems to be important to confirm diagnosis of CIDP in diabetic patients especially in presence of elevated protein content (> 1 g/l) and oligoclonal bands.

Screening for SACS gene causing ARSACS disease in Southern Italy

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Introduction: Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early-onset neurodegenerative disorder showing pyramidal, cerebellar progressive involvement and peripheral neuropathy. Believed at first to be restricted to Canada, ARSACS has been diagnosed in various countries afterwards. SACS, the gene responsible for ARSACS, maps to chromosome 13q11 and encodes for the protein sacsin. Sacsin is a 4,579-aminoacid protein, which may act as a chaperone. Material and methods: 23 patients with progressive early onset ataxia, pyramidal signs and clinical or neurophysiologic signs of peripheral neuropathy were selected. After informed consent direct sequencing of the nine coding exons and intron-exon boundaries was performed. Results: The complete sequencing of *SACS* in all patients detected three mutated patients and 12 different validated SNPs. We found a novel compound heterozygous mutation (c.5719C > T plus c.12628_12633delTGAAA, p.1907R>X plus p.4212R>X); a novel homozygous deletion (c.7249_7254delCAGAA, p.2426R > X); a novel homozygous deletion (c.700_702delAA, p.235K > X). All mutations cause premature truncation of sacsin and probably its loss of function. Moreover, we found a novel base pair change in one patient (c.1310C > T, p.437T > M) in heterozygous state. We cannot confirm the pathogenicity of this novel base pair change. Conclusions: Our results confirm the worldwide diffusion of ARSACS patients, the uniform clini-

cal presentation of the disease and the prevalence of loss of function mutations.

Infantile-onset Hereditary spastic paraplegia associated with *SPAST* mutations (SPG4): clinical and follow-up studies

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Introduction: Hereditary spastic paraplegia (HSP) associated with *SPAST* mutations (SPG4) is the most frequent form of autosomal dominant HSP accounting approximately for 40% of reported cases. The phenotype associated with HSP due to mutation in the spastin gene (SPG4) tends to be pure HSP with slowly progressing spasticity of the legs and hyperreflexia. Onset is mostly in the third to fifth decade but ample variability has been reported with congenital as well as late onset cases. Methods: We searched an ample series of families harboring SPAST mutation (over 100 patients) for SPG4 patients with infantile or congenital onset. We identified 12 patients with onset ranging from 0 to 5 years. We reviewed past information, and assessed their clinical condition by means of clinical examination and SPRS scale for HSP evaluation. Results: While clinical characteristics of infantile-onset SPG4 is apparently similar to more common adult-onset form, the progression of the disease was found to be significantly slower. Conclusions: Age of onset seems to be correlated with a significantly slower and more "benign" course of HSP as compared to classical adult-onset form. This result is clearly relevant for prognostic considerations as well as genetic counseling and could even reflect a different pathogenetic mechanism of *SPAST* mutations during fetal development and early infancy.

Muscle biopsy in diagnostic approach of subjects with POLG1 mutations

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Introduction: Mitochondrial encephalomyopathies are inherited disorders of oxidative metabolism, with a wide clinical, biochemical and genetic spectrum of expression, requiring a complex diagnostic flowchart. Mutations in the POLG1 gene compromise the stability of mtDNA and are responsible for numerous clinical presentations such as PEO (Progressive External Ophthalmoplegia), SANDO (sensory ataxic neuropathy, dysarthria and ophthalmoplegia), SCAE (spinocerebellar ataxia and epilepsy) and Alpers' syndrome. Material and methods: We carried out muscle biopsy in 8 patients with POLG1 mutations identified by sequencing. Seven mutations (679C > T, 752C > T, 803G > C, 1760C > T, 1943C > G, 2492A > G and 3527C > T) have been already described in literature, while three new mutations (347C > A, 2168G > T and 3076C > T) with a probably pathogenic rule were found. Clinical signs were heterogeneous and could include PEO, spastic tetraplegia, myopathy, polyneuropathy, ataxia, and parkinsonism. Mental retardation (MR) was present in 5 subjects. Results: On histological examination, only the 3 subjects without MR presented a typical picture of mitochondrial myopathy with ragged red fibers, COX deficient fibers and lipid accumulation. Among the patients with MR, COX deficient fibers and lipid accumulation were observed in 3 subjects, while 2 of them showed no significant alterations. It is interesting to note that 5/8 patients presented neurogenic signs with esterase positive fibers. At biochemical investigations, deficiency of one or more respiratory chain complexes was observed in the three subjects without MR and only in one of those with MR. Long-PCR showed multiple deletions of mtDNA in 2 subjects. Conclusions: Our results showed that muscle biopsy has a good predictive diagnostic value for mutations in the POLG1 gene in subjects without MR, while is less specific, or normal, in patients with MR, whose selection should be primarily done on the basis of the clinical signs.

High individual variability of levodopa pharmacokinetics in Parkinson's disease

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Introduction: Levodopa has been the mainstay of Parkinson's disease therapy for over 40 years. As such, most PD patients require levodopa-based therapy during the course of the disease. However, despite its proven efficacy, long-term levodopa therapy is associated with motor fluctuations, diskynesias and wearing-off phenomena. Aim of this study was to evaluate inter- and intra-individual variability of levodopa pharmacokinetic profiles and to correlate this profiles with clinical parameters. Material and methods: 30 patients with a diagnosis of idiopathic PD underwent a standard levodopa 250 mg test. Pharmacokinetics was performed on 3 non-consecutive days. Blood samples were collected at baseline and at 30, 60, 120, 180 min after levodopa administration. Plasmatic levodopa levels were analyzed by HPLC. Unified Parkinson's disease Rating Scale Part III was used to evaluate motor performances and fluctuations. Results: Pharmacokinetic profiles showed both inter-individual and intra-individual high variability of levodopa plasma levels. Moreover, no clear correlations between levodopa plasma levels and motor performances could be evidenced, regardless of the clinical stage. Conclusions: Our results confirm that levodopa pharmacokinetic is unpredictable and influenced by a variety of factors, mostly unknown. However, this high variability is not clearly correlated with fluctuations of motor performances.

Fractal analysis of retinal vessels in CADASIL and white-matter-lesion subjects

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Introduction: Fractal analysis of retinal vessels reveals altered branching in subject with CADASIL, as compared to matched control. The finding reflects reduced complexity, probably expression of the microvessel occlusions associated with the disease. In this study we sought to determine whether fractal analysis differentiates CADASIL-associated retinal changes from alterations potentially present in subjects with cerebral white matter lesions (WMLs). Material and methods: In order to test the hypothesis we carried out fractal analysis of retinal vessels in subjects with genetically tested CADASIL, in subjects who presented in our outpatient unit with clinical and neuroimaging features suggestive of CADASIL (WMLs), and in a control group. The study was, therefore, carried out in three groups: CADASIL (n = 11), WMLs (n = 11), and control (n = 11) subjects. Results: Mean fractal dimension (Mean-D) values (an index of retinal vessel branching complexity) were 1.38 ± 0.04 in CADASIL (mean ± SD), 1.42 ± 0.05 in WMLs no-CADASIL, and 1.45±0.04 in control group (p = 0.0001, CADASIL vs. control, Wilcoxon rank sum test). Conclusions: The preliminary results show a marked reduction of vessel branching complexity in CADASIL subjects, as compared to control, and, to a lower extent, as compared to WMLs subjects presenting with neuroimaging and clinical features suggestive of CADASIL.

Changes in the expression of calcium pumps in a rat embryonic striatum cellular model of Huntington's disease

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Introduction: Deregulation of calcium homeostasis can lead to neurodegeneration and seems to be involved in the ethiology of Huntington's disease (HD) [1]. To investigate whether the alteration of calcium homeostasis is an early event of neuronal degeneration in HD we studied the expression level of some calcium pumps in a cellular model of HD derived from rat embryonic striatum. We compared the steady-state level of sarco/endoplasmic reticulum (SERCA) and plasma membrane (PMCA) Ca^{2+}-ATPase in wild-type (STHdh+/STHdh+) and mutant (STHdhQ111/STHdhQ111) cell lines, both in undifferentiated and glial and neuronal differentiated conditions. Materials and methods: Cell lines were kindly provided by the University of Milan. Cell growth and cell differentiation were obtained as described by Cattaneo 1998 [2] and Ehrlich, 2001 [3]. Total proteins were extracted and used in Western blot assays to quantify the steady-state level of the indicated pumps. Commercial antibodies and β-actin expression were used for detection and normalization respectively. Results: Comparison between mutant and wild-type cell lines has shown that: 1. In the undifferentiated mutant cells SERCA2 protein concentration is significantly reduced while SERCA3 and PMCA1 protein concentrations are significantly increased. 2. in the glial differentiated mutant cells, PMCA1 and PMCA2 pumps are expressed at lower levels while in neuronal differentiated mutant cells PMCA3 pump is undetectable. Conclusions: Our study suggests that one of the earliest event indicating neuronal dysfunction in HD could be the changes in the expression of proteins that control calcium homeostasis like SERCA and PMCA. In the future, these pumps could represent early therapeutic targets in HD.

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Evaluation of oxidative stress markers in relation to physical exercise in amyotrophic lateral sclerosis patients

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Introduction: Amyotrophic lateral sclerosis (ALS), or Lou-Gehrig's disease, is a neurodegenerative disease that causes progressive degeneration of motor neurons in the motor cortex and anterior horn of the spinal cord. Among the assumptions made regarding the pathogenesis of ALS, the most reliable, call into question the excitotoxic theory, as well as the oxidative stress and alterations of mitochondria. Oxidative stress describes a condition in which the antioxidant defenses are unable to maintain cellular ROS levels below the toxicity threshold. This could be the result of an excessive ROS production, or a loss of the natural antioxidant defenses, or of both factors. Physical activity leads to a temporary imbalance between the ROS production and their disposal and, therefore, could be the primary cause of oxidative stress, however, there are currently major limitations of the knowledge of the relationship between oxidative stress and performance. The aim of this study was to investigate, through the monitoring of certain biological markers, some alterations of the mechanisms that underlie the regulation of cellular response against oxidative stress in relation to the ex-

ercise, in order to verify a possible correlation between exercise and increases in these parameters. Material and methods: The work was divided into two experimental phases: in the first phase were measured markers of oxidative damage in 32 ALS patients (mean age 63.6 ± 10.8) at diagnosis and in 54 healthy volunteers (mean age 69.9 ± 9.2) in order to check a possible alteration of the cellular state redox in patients compared to controls. Following discharge, 11 patients have conducted, for a period of 50 days, an aerobic workout of moderate intensity, while the remaining 21 have not been subjected to any training program. Then, the 11 patients who underwent training were called to perform an incremental exercise test on a cycle ergometer. The oxidative stress parameters were determinated in blood samples collected at baseline, for each increment of load, at exercise peak and 20 min after the end of the exercise. Results: The results concerning the analysis of peripheral markers of oxidative damage, confirm the redox alteration in ALS patients with sporadic form of diagnosis. Conclusion: What emerged from the comparison of these parameters before and after the training period is that these values have remained constant over time, while there has been an increase of oxidative damage in patients who received no training, and this leads us to conclude that, despite the aggressive and rapid progression of the disease, a moderate-intensity exercise may be helpful to maintaining the welfare of the musculoskeletal system.

AβPP (and its processing) regulate an intracellular signal pathway with a fast kinetic response and τ-phosphorylation

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The molecular mechanisms that cause AD are still unknown, and even the physiological function of the proteins genetically involved in the genesis of the disease (amyloid precursor protein and presenilins) is unclear. The leading theory in the AD field, the "amyloid hypothesis", foresees soluble Aβ peptides as the toxic species responsible for neurodegeneration, while gliosis and hyperphosphorylated NFTs represent secondary damaging events caused by Aβ [1]. Howevere new data challenged this theory [2] and in the last ten years it has been proposed a parallel theory, complementary to the amyloid hypothesis, in which AbPP, by acting as a cell surface receptor, modulates yet unclear cell signals, whose alteration may disrupt the neuronal homeostasis and cause neuronal impairment [3, 4]. We show that the C-terminal region of AβPP, modulates an intracellular signal based on the activation of both ERK1/2 and AKT which, in turn, modulates the phosphorylation of τ activating specific mitogenic phosphoepitopes. This event, which is normal in proliferating cells, has a fast kinetic response upon mitogenic stimuli and results in an abnormal and rapid phosphorylation on τ when levels of AβPP or of its CTFs are upregulated, as it may occur in Down Syndrome and in familial forms of AD. In these conditions, τ-hyperphosphorylation may be coupled to abnormal cell cycle re-entering of differentiated neurons and, ultimately, to cell death.

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Phenotypical and neuropathological features in a family with Frontotemporal dementia with the C9ORF72 hexanucleotide repeat expansion

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Introduction: Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis/motoneuron disease (ALS/MND) could represent the extremes of a clinical and neuropathological spectrum and a pathological hexanucleotide repeat expansion in the gene C9orf72 has been recently discovered as pathogenetic mechanism in familial FTD and ALS. The phenotypes associated to this mutation may be highly variable. This study was aimed to outline the clinical and neuropathological features of a pedigree bearing the pathological C9orf72 repeat expansion. Materials and methods: The family included 5 affected subjects in 2 generations. Genetic analysis was conducted in 4 patients. MAPT, PGRN, TDP-43, VCP and FUS genes were sequenced and GGGGCC hexanucleotide expansion in the first intron of C9ORF72 was screened by repeat-primed PCR. Neuropathological study was carried out on brain and cervical spinal cord in 2 patients. Results: Behavioral variant of FTD was diagnosed in the proband and relatives but no clinical signs suggestive for MND were developed. Mean age at onset was 58 ± 2 years and mean age at death was 67 ± 7 years. Pathogenic repeat expansions in C9ORF72 were detected in all examined patients. Neuropathological study showed marked cortical atrophy with spongiosis, loss and shrinkage of cortical neurons in frontal and temporal lobes and of motoneurons in anterior horn of spinal cord. Ubiquitin-positive neuronal and glial inclusions and dystrophic neurites associated with reactive astrogliosis were detected in pyramidal layers of frontal and tem-

poral cortical areas, hippocampus, subcortical structures and spinal and cranial motoneurons. Conclusions: We delineated phenotypic features in a family affected by FTD caused by C9ORF72 mutation suggesting a role for motoneuron vulnerability in FTD, even in absence of clinical MND.

Regulation of SIRT1 in Duchenne Muscular Dystrophy by miRNA-34a

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SIRT1 (silent information regulator 1) is a protein that belongs to the family of sirtuins (SIRT1-SIRT7), the Class III protein deacetylases that use NAD+ as a cofactor in such a way their activity is modulated by NAD+/NADH ratios. It involved in metabolic processes, in response to stress and cell aging. SIRT1 regulates the activity of several transcription factors and cofactors reshaping the chromatin structure, by deacetylation of histones, and modulating a large number of non-histone substrates such as transcription factors p53, NF-κB, FOXO. Recent studies performed on mdx mice, an animal model of Duchenne muscular dystrophy (DMD), identified that SIRT1 is a probable useful strategy to treat this disease. DMD is inherited myogenic disorder accompanied by progressive skeletal muscle weakness and degeneration, probably due to the limited capacity of satellite cells (precursors of myoblasts) to proliferate. Is useful to understand what are the regulatory mechanisms that control the expression and activity of the SIRT1 in satellite cells of DMD muscle, so as to be able to identify a possible gene therapy that induces the myoblast to differentiation. This study aims to investigate, in DMD muscle, expression of SIRT1, miR-34a and several mRNAs coding for mTOR and Ras/Raf/MEK/ERK signaling pathway proteins afar controlled by SIRT1. In addition, selected proteins will be further validated as potential targets of miR-34a by expression studies, such as immunohistochemistry, real-time PCR and Western blot. Our preliminary results show an expression of SIRT1 in tissues of DMD myoblasts. Furthermore, it was found that there are no significant differences in expression of mTOR between the DMD biopsy specimens and samples control (muscles without alterations).

Frequency of the chromosome 9 hexanucleotide repeats in Italian patients with frontotemporal lobar degeneration

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Introduction: A hexanucleotide repeat expansion in the first intron of *C9ORF72* has been shown to be responsible for a high number of familial cases of amyotrophic lateral sclerosis or frontotemporal lobar degeneration (FTLD) with or without concomitant motor neuron disease (MND) phenotype and TDP-43 based pathology. Objectives: To analyze the frequency of the hexanucleotide expansion in a population of patients with FTLD (with or without MND). Population and methods: DNA from 704 patients with FTLD (24 with concomitant MND) were collected. Analysis was done by repeat-primed PCR and sequencing. Results: At present, 563 samples have been screened. 30 were carriers of the mutation (5.5%). 21 of them were diagnosed clinically with behavioural variant frontotemporal dementia (bvFTD), 7 with bvFTD-MND (diagnosed initially with bvFTD and developing MND over time) and two with semantic dementia. Conclusions: the hexanucleotide expansion in chromosome 9 is likely a common cause for FTLD, together with progranulin and τ-mutations. Patients' follow-up is ongoing to clarify whether the presence of this genetic defect is a prognostic biomarker predicting the development of MND in patients diagnosed with bvFTD.

Neuroglobin: a new protein involved in estrogen protective effects in neuronal cells

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Introduction: Neuroglobin (Ngb) is the first nerve globin identified in neuronal tissues of humans. Ngb-overexpression drastically reduces the size of cerebral infarct and enhances cell survival in Alzheimer's disease, anoxia, and oxygen deprivation. All these data indicate a protective function of Ngb in the brain. Unfortunately, Ngb does not cross cell membranes, thus direct administration of Ngb is not a feasible therapeutic strategy. A significant contribution to the therapeutic potential of Ngb could derive from the identification of drugs and endogenous modulators that stimulate the expression of Ngb. The steroid hormone 17β-estradiol (E2) is a good candidate due to its variety of actions on the brain which include protection against neurotoxicity and neurodegeneration. Results: Here, we report our recent data demonstrating that 17β-estradiol (E2) rapidly induces 300% increase of Ngb levels in several neuronal cells. Ngb is part of the E2 protection against H_2O_2-induced toxicity. In fact, E2 pre-treatment decreased caspase-3-dependent apoptosis induced by H_2O_2 exposure. This E2 effect against H_2O_2 toxicity requires Ngb and the signal transduction pathways triggered by estrogen receptor-β subtype. Upon E2 stimulation, Ngb localize mainly into mitochondria where the physical association with the mitochondrial cytochrome *c* occurs. H_2O_2 insult further increased the Ngb:cytochrome *c* association reducing its release into the cytosol. As a consequence, a decrease of caspase-3 activation and, in turn, of the apoptotic cascade

activation occurs. Conclusions: Experiments performed in other models confirm these results highlighting the widespread involvement of ERβ-Ngb in E2-induced neuroprotection.

Physicochemical properties of Aβ peptides in cerebrospinal fluid of Alzheimer's disease patients

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Introduction: Cerebrospinal fluid (CSF) biomarkers are currently included among supportive criteria for Alzheimer's disease diagnosis (AD). In particular, a decrease of CSF levels of amyloid beta peptide 1-42 (Aβ42) coupled with an increase of τ and phospho-τ_{181} protein levels support AD diagnosis. In this study we evaluated the diagnostic value of ELISA and Western blot analysis in determining Aβ40 and Aβ42 levels. Materials and methods: We analyzed CSF samples from 40 patients with diagnosis of dementia and from 15 age matched controls. CSF samples were tested by using two different ELISA kits one from Innogenetics (Ghent, Belgium) and the other from Wako (Osaka, Japan), and by Western blot. Results: ELISAs provided different results in the levels of Aβ42 peptides. Spurred by these discrepancies, we determined the Aβ40 and Aβ42 levels by Western blot analysis. While in CSF samples, Aβ40 values overlapped those obtained by ELISA, western blot analysis showed Aβ42 values significantly higher, indicating that ELISA detects only partially Aβ42. Conclusions: These findings indicate that part of Aβ42 peptides in the CSF exist under oligomeric/polimeric forms or in combination with other proteins, thus masking reactive epitopes under the ELISA assay. This study is supported in part by Fondazione Cariverona.

Nuclear localization of phosphodiesterase 10A (PDE10A) in the R6/2 mouse striatal interneurons

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Introduction: Cyclic nucleotides play an important role as second messengers in the CNS. Intracellular concentrations of cAMP and cGMP are modulated by the rate of degradation by a variety of phosphodiesterases (PDEs). PDE10A is the single member of one of the newest PDE gene families. PDE10A has been observed in the brain mostly in the striatal projection neurons [1]. However, we have previously observed [unpublished data], in the striatum, a number of PDE10 immunoreactive neurons that were not projection neurons. Methods: R6/2 mice and their wild type littermates were sacrificed at 5, 9, 13 weeks of age, and single and double label immunohistochemistry were performed to identify the different neuronal subtypes of the striatum (medium spiny, cholinergic, parvalbuminergic, somatostatinergic). Results: PDE10A was observed in all subtypes of striatal neurons. In the spiny projection neurons, PDE10A localized in the cytoplasm, whereas in the striatal interneurons, regardless of the subtype, PDE10A displayed a clearly nuclear localization. This was true both for the wild type and for the R6/2 mice. Conclusions: Our study demonstrates that PDE10A is contained not only in the medium spiny neurons, but also in the striatal interneurons. Moreover, the different compartmentalization might be explained by a different activity exerted by PDE10A between projection neurons and interneurons.

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Melatonin, aging and chronic degenerative diseases. The Treviso longeva (Trelong) study

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Introduction: It has been reported a compromised ability of the pineal to produce melatonin nightly in elderly humans and that reduced melatonin levels may also be a risk factor for cancer. The purpose of this study was to evaluate the relationship between melatonin, aging and chronic degenerative diseases in the sample of Trelong study. Material and methods: The Trelong study started in 2003. Urinary 6-sulfatoxymelatonin (aMT6s), that serves as a useful tool for the estimation of serum-melatonin secretion, was assayed in urine of 260 survivors aging subjects collected in 2010 follow-up and stored at –80 °C by using an enzyme-linked immunosorbent assay (ELISA) kit (product 01-EK-M6S, ALPCO Immunoassays, Windham, NH). All aMT6s levels were creatinine standardized ((aMT6s)/(creatinine)). Results: Age (77 – 105 years) mean 85.3 ± 6.3; sex, n (% female) 146 (56.2%); aMT6s 44.2 ± 42.7 ng aMT6/mg creatinine. Melatonin levels tend to have an association with aging and to decline more in males than in females (40.5 vs. 47.0), but both not significantly. Melatonin levels are significantly lower in patients reporting insomnia ($p < 0.03$). There is a significant inverse correlation between melatonin levels and the disease count index (DCI) ($p < 0.05$). The new cases of cancer, after the baseline measurement, tend to have average levels of melatonin lower than in those without cancer (35.6 vs 44.6) even if the difference is not significant. The combination of new cases of cancer with new cases of cerebrovascular disease ($n = 24$) show lower levels of melatonin compared to those without these two diseases (35.1 vs. 44.9) ($p = 0.08$). Conclusions: The onset of chronic diseases, after baseline measurement, is still too low to provide meaningful results, but suggests that cancer and

cerebrovascular disease are possible candidates for an association with melatonin levels. The association with sleep disorders is confirmed. These early findings would confirm the protective role of melatonin against various chronic diseases.

Expression of the chemokine receptors CXCR4/CXCR7 and their ligands in human meningioma: localization and potential role in tumor microvasculature

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Introduction: Chemokines are key-players in CNS physiology and pathology. Particularly, CXCL11 and CXCL12 and their receptors CXCR4 and CXCR7 are involved in brain tumor cell proliferation, invasion and angiogenesis. Material and methods: The expression of CXCL11, CXCL12, CXCR4, and CXCR7 in 22 human meningiomas (11 WHO I, 11 WHO II/III) was evaluated at the mRNA level by quantitative RT-PCR and at protein level by confocal immunofluorescence on the corresponding paraffin-embedded sections. Results: Quantitative analysis of CXCR4/7 and CXCX11/12 mRNA revealed their expression in all tumor samples at various levels. CXCR7 and CXCL12 mRNA amount was significantly related to both proliferative activity, as evaluated by the proliferative index MIB-1, and tumor aggressiveness, being median mRNA values in WHO II/III higher than Grade I tumors. We found significant correlations between the expression of CXCL12-CXCR7, CXCL12-CXCL11 and CXCL11-CXCR7 pairs. Immunofluorescence experiments exhibited that CXCR4 and CXCL12 were highly expressed within all meningiomas while CXCL11 and CXCR7 showed a focal immunopositivity in clustered tissue areas and CXCR7 was mainly detectable in vascular endothelium. Double staining for endothelial markers (CD31 and CD34) and CXCR7 showed that co-expression occurred in both microvasculature and mature vessels. CXCL11-expressing perivascular cells were identified in desmin-positive pericytes. Preliminary *in vitro* results showed that CXCL12 and CXCL11 increased proliferation and tube formation of endothelial-cells derived from fresh meningioma tissues, likely involving CXCR7 interaction. Conclusions: These results highlight the preferential CXCR7 and CXCL12 expression within more aggressive tumors and the possible role of CXCR7 in meningioma vascularization.

Ruta graveolens aqueous extract inhibits proliferation of undifferentiated neural cells and induces differentiated neurons re-entry in cell cycle

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Introduction: In CNS, aberrant proliferation causes cancer and impaired survival of differentiated neurons induces neurodegenerative disorders. In order to find novel therapeutic targets able to inhibit aberrant cell proliferation and/or enhance differentiated cells survival, we analyzed properties of aqueous extract of *Ruta graveolens* on differentiated and proliferating neural cells. *Ruta g*. is currently used for its diuretic, sedative, and analgesic effects and recent studies described antiproliferative effects on different cancer cells. Materials and methods: We used a mouse mesencephalic embryonic cell line, A1 mes-c-myc cells (A1) that are proliferating/undifferentiated in the presence of serum. They cease to proliferate and differentiate when serum is withdrawn and cAMP is added. Aqueous extracts (*Ruta g*. a.e.) were obtained from young leaves chopped, infused in boiling water and lyophilized. Extract concentrations of 10 mg/ml, 1 mg/ml and 0.1 mg/ml were tested. Cell counting was performed by MTT assay and Trypan blue method. Cell cycle was analyzed by cytometry after PI incorporation. Cell signalling was analyzed by western blotting. Results: *Ruta g.* a.e. inhibits A1 cells proliferation and induces increase in ERK phosphorylation. In presence of the ERK pathway inhibitor, PD, *Ruta g.* a.e. is unable to induce cell death indicating that ERK is involved in the *Ruta g*. effect on A1 proliferating cells. On the other hand, when *Ruta g*. a.e. is added, the number of differentiated A1 cells appears significantly higher as compared to control conditions and the analysis of the cell cycle showed an increased number of cells in G2/M phase in differentiated cells treated with *Ruta g.* a.e. Conclusions: *Ruta g*. a.e. could represent an interesting therapeutic tool since it is able at the same time to inhibit undifferentiated cell proliferation and to induce re-entry in the cell cycle of differentiated neurons.

Leukoencephalopathy with thalamus and brainstem involvement and high lactate caused by EARS2 mutations

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Introduction: Multiple defects of mtDNA-related complexes of the mitochondrial respiratory chain (MRC) is a frequent biochemical signature of a large group of genetically undetermined infantile-onset mitochondrial encephalo(myo)pathies. Methods: To identify responsible genes, we used exome-next generation sequencing in a selected cohort of patients with multiple MRC defects. Results: In a singleton baby boy we found two mutant alleles for *EARS2*, the gene encoding the mitochondrial glutamyl-tRNA synthetase. The MRI was hallmarked by extensive symmetrical cerebral white matter abnormalities sparing the periventricular rim, and symmetrical signal abnormalities of the thalami, midbrain, pons, medulla oblongata, and cerebellar white matter. [^{1}H]-MRS showed a lactate peak in the affected areas. We matched this MRI pattern with the MRI pattern of a cohort of 11 previously selected unrelated cases, and found *EARS2*

mutations in all of them. Subsequent detailed clinical and MRI follow-up revealed two distinct groups: mild and severe. All 12 patients shared an infantile onset and rapidly progressive disease with severe MRI abnormalities and increased lactate in body fluids and by [^{1}H]-MRS. However, whilst patients in the "mild" group partially recovered and regained milestones in the following years with striking MRI improvement and declining lactate levels, those of the "severe" group were characterized by clinical stagnation, brain atrophy on MRI and persistent lactate increase. Conclusions: A new neurological disease, early-onset leukoencephalopathy with thalamus and brainstem involvement and high lactate, LTBL, is hallmarked by unique MRI features, defined by a peculiar biphasic clinical course, and caused by mutations in a single gene, *EARS2*, expanding the list of medically relevant defects of mtDNA translation.

Human CSF amyloid-β peptides exosomal compartmentalization in Alzheimer's disease

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Introduction: The predominant protein component of Abeta plaques in Alzheimer's disease (AD) are strongly aggregating peptides with an approximate molecular mass of 4 kDa. Among these peptides, $A\beta_{1\text{-}40}$ and $A\beta_{1\text{-}42}$ have been the dominant focus research, but it is well established that N- and C-terminally truncated or modified forms of Aβ peptides also exist in AD brain and cerebrospinal fluid (CSF). Herein, we investigated the Aβ CSF exosomal compartmentalization of N and C-terminally truncated Aβ peptides in patients with AD and with subjective memory complaints (SMCs). Materials and methods: Microvesicles released by human CSF (AD: n = 10; SMCs n = 10) were isolated by ultracentrifugation and sucrose gradient fractionation. The immunoproteomic analysis for truncated Aβ peptides detection was performed using SELDI-TOF mass spectrometry on PS20 chip array and specific monoclonal antibodies (6E10 + 4G8). Results: We observed i) that exosomes transport 14 different Abeta peptides (including 3 N-terminally truncated forms); ii) a differential exosomal Abeta compartmentalization in health and disease. Conclusions: A better understanding of the mechanisms involved in exosomal Abeta processing, release, and uptake is of great therapeutic interest and may have important implications for the fight against AD.

A case of Multiple Sclerosis with pure, massive superficial demyelination

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Introduction: Although multiple sclerosis has been classically regarded as a primarily white matter disorder, recent immunohistochemical studies with antibodies against myelin antigens have shown that the grey matter may be also heavily affected. Case report: A 60-year-old man presented with generalized weakness, and sensory disturbances at the left leg. Four months after the onset, walking was impossible and the patient died 16 months later. CSF and MRI were not suggestive of multiple sclerosis. At neuropathological examination, although no significant areas of demyelination were detected in the brain by routine myelin stainings, immunohistochemistry for myelin basic protein showed the presence of widespread superficial myelin loss, that appeared as a continuous band of unstained neuropil lining the pial surface and the ventricular walls in the cerebral hemispheres. Subpial demyelination involved also the cerebellum and the optic nerves. The neuropathological diagnosis of multiple sclerosis was based also on the involvement of the spinal cord where myelin loss affected both the grey and the white matter. Conclusions: Our findings highlight that subpial myelin loss may occur independently from classic plaques in the cerebral white matter and may be a pathogenetically relevant event at least in a subset of patients with multiple sclerosis.

Beneficial effects of peripherally administered brain derived neurotrophic factor (BDNF) in the R6/2 mouse model of Huntington's disease

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Introduction: In HD, mutated huntingtin causes a major loss of brain derived neurotrophic factor (BDNF), causing striatal atrophy. In fact, BDNF, essential for the survival of the striatum, is transported anterogradely from the projecting cortical neurons. BDNF has been described not to cros the blood-brain barrier. However, Schmidt and Duman [1] showed that peripheral administration of BDNF increases hippocampal and striatal expression of BDNF in a major depression rat model. Thus, we aimed at increasing BDNF levels in the striatum by administering recombinant BDNF to the R6/2 mouse model of HD. Methods: Alzet micropumps filled with the recombinant BDNF (Regeneron) or saline, were placed in R6/2 mice and their wild type littermates and sacrificed at 5, 9, 13 weeks of age, and histology, single and double label immunohistochemistry and Western blotting were performed. Results: Survival and neurological signs were significantly improved by recombinant BDNF in the R6/2 mice. Moreover, striatal atrophy was reduced. Striatal protein level of BDNF and mRNA were both increased, compared to the saline group, in the R6/2 mice treated with recombinant BDNF. Conclusions: Our study demonstrates that peripherally-administered BDNF is beneficial in the primary and secondary outcome measures in the R6/2 mouse model of HD. Moreover, peripheral BDNF represents a positive feedback for BDNF production in the brain, and thus it should be considered as a therapeutical approach to fight HD.

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A novel globin discovered in the brain: its evolutionary and physiological significance

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Introduction: Neuroglobin (Ngb) is a member of the hemoglobin superfamily, predominantly expressed in neurons and endocrine cells. The over-expression of Ngb protects neurons against β-amyloid, nitric oxide, and hydrogen peroxide toxicity *in vitro* and attenuates histopathological and behavioural features in a murine model of Alzheimer's disease *in vivo*. These findings highlight the protective role played by Ngb over-expression against neurodegeneration. The finding that colorless-blooded Antarctic icefishes (family Channichthyidae, suborder Notothenioidei) retain the Ngb gene despite the loss of hemoglobin and myoglobin in most species, may have important implications in the physiology of the brain and may help the elucidation of Ngb function. Methods: Cloning and sequencing of cDNA; expression and purification of Ngbs; laser-flash photolysis; classical molecular dynamics. Results: Red-blooded *Dissostichus mawsoni* and icefish *Chaenocephalus aceratus* Ngb cDNAs from retina and brain, respectively, were cloned, expressed in *Escherichia coli* and the recombinant proteins were purified. Their structural and functional characterisation was performed by spectroscopic and kinetic measurements, and dynamic simulation. Conclusions: The icefish, lacking the globins common to most vertebrates, is a suitable model to enhance the knowledge on the function of globins in the brain and retina. Comparison of structural and functional properties suggests that the two Antarctic fish Ngbs preserved the function proposed for human Ngb. The peculiarities showed by these proteins are a new challenge for the interpretation of the structure/function relationship.

Screening for ABHD12 gene causing PHARC disease in Southern Italy

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Introduction: Polyneuropathy, hearing loss, ataxia, retinitis pigmentosa, and cataract (PHARC) is a neurodegenerative disease characterized by early onset and involvement of central and peripheral nervous systems. PHARC was initially reported as a Refsum-like disease and *ABHD12* was identified as the causative gene. *ABHD12* codifies for a serine hydrolase with a role in the endocannabinoid metabolism. Material and methods: Twelve patients were selected for mutational analysis according to the following criteria: recessive inheritance, early onset, ataxia and ocular impairment (retinitis and/or cataract or optic atrophy). After informed consent direct sequencing of the 13 coding exons and the intron-exon boundaries of *ABHD12* gene was performed. Results: Mean age at onset was 19.12 ± 16.09. All had gait ataxia and ocular involvement, three hearing loss, three neuropathy. Mild-to-moderate hemispheres/vermis atrophy was present at MRI in 5 patients. No mutations were found but ten validated SNPs were identified in our population. One SNP, rs746748G/A in exon 12, was non synonymous: it was present in heterozygous state in two patients. An heterozygous deletion in the 3'-UTR, c.*324delG, not reported, was found in two patients; it seems not to be pathogenic. Conclusions: Although 19 PHARC patients from Norway, Algeria, Emirates and USA have been so far described, PHARC is not present in our selected patients from Southern Italy.

Epidemiology of pediatric primary tumors of the nervous system: A retrospective study of 633 cases from a single Moroccan institution

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Introduction: There are several reports regarding the epidemiology of pediatric brain tumors. However, little is known about the profile of pediatric brain tumors in Africa and in Morocco in particular. The authors report the results of epidemiological analysis of a retrospective review of childhood primary brain tumors in a single institution. Methods: A retrospective review of all cases of primary brain from 1 month to 15 years diagnosed at the Department of Pathology of the Hospital of Specialities of Rabat between January 1991 and December 2009 was performed. Results: 633 primary tumors of the central nervous system were reviewed with a mean of 33.31 cases per year. According to the gender, 55% of the tumors occurred in males and 45% in females. The mean age was 8.36 years. Of all the tumors, 47% were situated in the supratentorial compartment, 48% in the infratentorial compartment, and 5% in spinal cord. In the infratentorial compartment, 82% of tumors are located in the cerebellum, 15% in the fourth ventricle, 2% in the brain stem and 1% in the cerebellar pontine angle. In the supratentorial compartment, two third of the tumors were located in the cerebral hemispheres and the sellar region. Thus 39% of tumors are located in the cerebral hemispheres followed by the sellar/suprasellar region (30%), lateral and third ventricles (11%), pineal region (8%), meninges (5%), choroid plexus (4%), and optic chiasma/tracts (3%). The most common types of tumors diagnosed were pilocytic astrocytoma and medulloblastoma together accounting for nearly half of the cases (46%), followed by craniopharyngiomas (9%), ependymomas grade II (6.5%), glioblastomas (6%), astrocytomas Grade II (4.4%), ependymomas Grade III (3.9%). The other tumors represent 22.6%. Conclusions: We think that our results reflect

fairly well the incidence of tumors of the nervous system in children due to the fact that this study was performed through many years in a single institution with a homogeneous neuropathological approach.

Phosphodiesterase 10A (PDE10A) localization in the R6/2 mouse model of Huntington's disease

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Introduction: In Huntington's disease (HD) mutant huntingtin protein impairs the function of several transcription factors, in particular the cAMP response element-binding protein (CREB). CREB activation can be increased by targeting phosphodiesterases such as phospohodiesterase 4 (PDE4) and phosphodiesterase 10A (PDE10A). Indeed, both PDE4 inhibition [1] and PDE10A inhibition [2] proved beneficial in the R6/2 mouse model of HD. However, Hebb et al. [3] reported that PDE10A decline in R6/2 mice. These findings raise the issue of how PDE10A inhibition would be beneficial in HD if such enzyme is decreased. Methods: R6/2 mice and their wild type littermates were treated with the PDE10A inhibitor TP10 (Pfizer) or saline, sacrificed at 5, 9, 13 weeks of age, and single and double label immunohistochemistry and western blotting were performed. Results: PDE10A increased in the spiny neurons of R6/2 with the progression of the disease. In the striatal cholinergic interneurons, PDE10A was lower and it did not change with disease progression. In the TP10 treated R6/2, PDE10A levels were lower than in the saline group in all subsets of striatal neurons and also in the cortex. Conclusions: Our study demonstrates that PDE10A is increased in the spiny neurons of R6/2 mice striatum, thereby explaining the beneficial effects of PDE10A inhibition in this model. Moreover, neurons that are less vulnerable to HD contain lower levels of PDE10A.

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Biomarkers of angiogenesis in 52 pediatric glial brain tumors

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Introduction: Malignant gliomas in children are very rare, comprising 5 – 10% of childhood intracranial neoplasms. Glioblastoma and pilocytic astrocytoma showed similar vascular pattern but turnover of endothelial and tumor cells were lower in pilocytic astrocytoma. Pilocytic astrocytoma is the most frequent brain tumor in children, although highly vascular generally is circumscribed and slowly growing tumor, corresponding to Grade I (WHO 2007). Little has been reported on characterization of pediatric GBM, which histological features were similar to that of adult GBMs. Aim of our study was to characterize tumor vasculature and angiogenic profile of pediatric glial tumors to giving potentially new insight in targeting tumor angiogenesis. Materials and methods: We performed an immunohistochemical study on 52 pediatric patients, which underwent surgery in our Institute. They were: 40 pilocytic astrocytomas and 12 glioblastomas. We analyze the expression of: PV-1, Cav-1, endoglin (CD105), VEGF, PDGFR-α and WT-1. Results: We found PV-1 and Cav-1 expressed in neoplastic endothelial cells. Endoglin expression showed a significant correlation with microvessels density and seems to be a marker of activated endothelium, expecially in pilocytic astrocytoma with higher vascular proliferations. In our glioma patients we observed a significant different expression of WT1 and PDGFR-α in endothelial and neoplastic cells, related to malignancy grade expression in tumor cells was correlated to grade of malignancy. VEGF was expressed in all glioblastomas. Conclusions: The different expression profiles of these endothelial markers can help to evaluate the different mechanisms of angiogenesis in pediatric glial tumors, suggesting new prognostic markers and potential therapeutic targets.

Concurrent mutations in AFG3L2 and paraplegin in patients with spinocerebellar degeneration

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Introduction: We recently showed that *AFG3L2* mutations cause dominant ataxia SCA28 [1]. AFG3L2 and its partner protein paraplegin, which causes recessive spastic paraplegia SPG7, are components of the mitochondrial *m*-AAA complex involved in protein quality control. Since yeast functional studies had showed that paraplegin coexpression can complement AFG3L2 mutations in some cases, we investigated the possible coinheritance of *AFG3L2* and *SPG7* mutations in patients with spinocerebellar syndromes. Results: We identified 3 probands with double heterozygosity for AFG3L2 and paraplegin mutations. Two ataxic patients carry an AFG3L2 mutation along with paraplegin^{A510V}. The third proband carries a *de novo* AFG3L2 mutation in the highly conserved SRH region of the ATPase domain along with the inherited deletion of 3 SPG7 exons. This patient exhibits early-onset optic atrophy and a L-dopa-responsive spastic-ataxic

syndrome with extrapyramidal signs. A muscle biopsy revealed an isolated complex I deficiency. Evaluation of substrate processing in patient's fibroblasts showed abnormal processing of OPA1. Conclusions: Our data indicate that the presence of a loss-of-function mutation in paraplegin may act as a disease modifier for heterozygous AFG3L2 mutations. Concurrent mutations in both components of the *m*-AAA complex may result in a complex phenotype, thus expanding the clinical spectrum of AFG3L2-associated mutations. Moreover, biochemical and cell biology studies revealed a crucial role of the complex in the processing of OPA1 and the maintenance of mitochondrial morphology and dynamics. (Telethon grant GGP09301 to FT).

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Diffusion indeces variations in different transgenic models of Alzheimer's disease detected with diffusion tensor imaging

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Introduction: In addition to the classical lesions in the gray matter, Alzheimer's disease (AD) features damage to the white matter (WM). This can be identified using diffusion tensor imaging (DTI). In the frame of IMI-PharmaCog consortium we compared the changes in DTI parameters obtained with hand drawn regions of interest (ROIs) with a whole brain voxelwise method in 3 different mouse models of age-dependent amyloid deposition. Methods: Wild-type, single APP mutant, PDAPP, double APPxPS-1 mutant TASTPM, and triple APPxPS-2x-τ mutant mice were followed from 3 to 20 months. The acquisitions were gained using DTI EPI sequence. Manual analysis: Different ROIs were manually drawn using the color coded map. *Voxelwise analysis.* We developed a processing pipeline based on a modified version of the Tract Based Statistical Analysis protocol (TBSS) [1]. Results: FA reduction was seen in several brain structures of older TASTPM when compared with age-matched WT. These results have been confirmed using manual and automated methods. The same areas in single and triple mice were substantially unchanged with aging. Conclusions: Our study detected age-related WM deficits in TASTPM mice which could be a potential model to study WM pathology in AD. Automated method showed that FA decreases in regions not considered with manual analysis suggesting greater sensitivity of automated methods. The research leading to these results was conducted as part of the PharmaCog consortium funded by the European community's seventh framework programme for the innovative medicine initiative under grant agreement n°115009 (www.alzheimer-europe.org).

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Cerebrospinal fluid levels of Aβ-42 relationship with cholinergic cortical activity in Alzheimer's disease patients

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The dysfunction of cholinergic neurons is a typical hallmark in Alzheimer's disease (AD). In animal models of AD, fragments of amyloid beta protein (Aβ) and τ-protein are thought to interfere with central cholinergic transmission, specifically with synthesis and release of acetylcholine. Thus, we aimed to investigate whether the cerebrospinal fluid (CSF) levels of $A\beta_{1-42}$ and τ-proteins in AD patients could influence physiological central cholinergic activity. In AD patients (n = 27), central cholinergic function was evaluated *in vivo* by using Short Afferent Latency Inhibition (SLAI), and compared to age-matched healthy controls. In the same AD patients CSF samples were collected through lumbar puncture in order to obtain individual levels of $A\beta_{1-42}$, total τ (t-τ) and phosphorylated τ (Thr181). SLAI was decreased in AD patients in comparison to age-matched healthy controls. We found that in patients there was a negative correlation between the individual amount of cholinergic activity assessed by SLAI and the CSF levels of $A\beta_{1-42}$. On the other hand, there was a positive correlation between the levels of SLAI and CSF p-τ. No correlation was found when SLAI was analyzed together with t-τ. These results demonstrate that mechanisms of cortical cholinergic activity are altered in patients bearing a pathological CSF hallmark of AD, suggesting that these peptides may have some influence on the cholinergic dysfunction in AD. We suggest that coupling of CSF biomarkers with neurophysiological parameters of central cholinergic function could be important to better detect ongoing mechanisms of neural degeneration *in vivo*.

Plasmin system of Alzheimer's disease patients: CSF analysis

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Alzheimer's disease (AD) is a multifactorial neurodegenerative disorder characterized by the extracellular deposit of amyloid beta (Aβ), manly of the amyloid $beta_{1-42}$ ($A\beta_{1-42}$) peptide in the hippocam-

pus and neocortex and leading to progressive cognitive decline and dementia. The possible imbalance between Aβ production/degradation process was suggested to contribute to the pathogenesis of AD. Among others, the serine protease plasmin has shown to be involved in $A\beta_{1-42}$ clearance, hypothesis strengthened by neuropathological studies on AD brains. To what extent changes observed in brain tissue can also be detected in the CSF, and could be used as a diagnostic tool in AD *in vivo* has never been determined. To do this, we analyzed CSF samples from AD and aged matched controls, looking at plasminogen, tissue plasminogen activator (tPA) and plasminogen activator inhibitor (PAI-1) protein levels and t-PA and urokinase plasminogen activator (u-PA) enzymatic activities. We also measured $A\beta_{1-42}$, total τ and phospho-τ_{181} CSF levels and sought for possible relationship between them and plasmin system values. Our findings showed that t-PA, plasminogen and PAI-1 levels, as t-PA enzymatic activity, remained unchanged in AD respect to controls; u-PA activity was not detected. We conclude that CSF analysis of plasminogen system do not reflect changes observed post-mortem. Unfortunately, CSF detection of plasmin system could not be useful biomarker for either AD diagnosis or disease progression. However, these findings do not exclude the possible involvement of plasmin system in AD.

RNA polymerase III drives alternative splicing of the potassium channel interacting protein contributing to brain complexity and neurodegeneration

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Introduction: Alternative splicing generates protein isoforms that are conditionally or differentially expressed in specific tissues. Discovery of factors that control alternative splicing might clarify the molecular basis of biological and pathological processes [1]. Materials and methods: We investigated the function of a novel non-coding RNA in neuroblastoma cells geticaly engineered to overexpressed and/or downregulate the molecule of interest. Results: We found that IL1-a dependent upregulation of 38A, a small RNA polymerase III-transcribed RNA, drives the synthesis of an alternatively spliced form of the Potassium Channel Interacting Protein (KCNIP4). The alternative KCNIP4 isoform cannot interact with the γ-secretase complex, resulting in modification of γ-secretase activity and Amyloid Precursor Protein processing and increased secretion of beta-amyloid enriched in the more toxic Abx-42 species. Notably, synthesis of the variant KCNIP4 isoform is also detrimental to brain physiology because it results in the concomitant blockade of the fast kinetics of potassium channels. This alternative splicing shift is observed at high frequency in tissue samples from Alzheimer's disease patients. Conclusions: Our results suggest that RNA polymerase III co-genes may be upstream determinants of alternative splicing that significantly contribute to homeostasis and pathogenesis in the brain [2].

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Cerebral structural changes in different transgenic models of Alzheimer's disease with magnetic resonance imaging

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Introduction: The atrophy of several brain regions determined by magnetic resonance imaging (MRI) analysis has been associated with Alzheimer's disease (AD). In this study we performed longitudinally MRI analysis in wild type (WT) and three different transgenic mice to investigate structural changes associated to β-amyloid cerebral deposition. Methods: We performed high resolution (146 × 117 × 146 μm voxel size) in vivo MRI with a 7T Bruker Biospec system. We extracted T2 brain maps using a multi-slice multi-echo T2 sequence in single APP mutant, PDAPP, double APPxPS-1 mutant TASTPM, and triple APPxPS-2xtau mutant. Mice were followed form 4 to 24 months. The volume measurement was obtained manually. Results: In the three models the hippocampus, striatum and frontal cortex show a similar structural modification throughout aging. The hippocampal volume was substantially unchanged, frontal cortex exhibited a reduction of the volume while a atrophy of striatum was evident. The progressive atrophy started in the cortex at 13 months in both TASTPM and TAUPS2_APP mice, while in PDAPP mice it occurred earlier. An increase in ventricles volume with aging was found in PDAPP and TASTPM mice. Conclusions: Our findings suggest that these models are potentially relevant in the translational study of the effects of progressive Aβ deposition. From the comparison we found that the models have in common the strong progressive atrophy of the striatum. The research leading to these results was conducted as part of the PharmaCog consortium funded by the European Community's Seventh Framework Programme for the Innovative Medicine Initiative under

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C9ORF72 hexanucleotide repeat expansions in ten patients with amyotrophic lateral sclerosis (ALS) from Campania: analysis of genotype-phenotype interaction

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Introduction: Recent neurobiological findings suggest that amyotrophic lateral sclerosis (ALS) is a multisystem disease with both motor and extramotor impairment. Remarkably, ALS can co-occur with any of the Frontotemporal Dementia (FTD) clinical subtypes, but is most commonly associated with the behavioral variant (bvFTD). Moreover, it has been recently reported that a large proportion of patients with familial ALS (FALS) and FTD are associated with a hexanucleotide (GGGGCC) repeat expansion in the first intron of *C9ORF72* [1, 2]. Material and methods: We have recruited 70 consecutive ALS cases between 2010 and 2011 to be screened for mutations of *C9ORF72* gene. Results: Ten ALS patients (14.2%) carried the pathogenic repeat expansion, 7 of which had FALS and 3 sporadic ALS (SALS). None of the 70 regionally-matched control samples (140 chromosomes) carried the mutation. Five FALS patients had bvFTD in addition to ALS, whilst 2 FALS and all SALS cases had only a mild frontal dysfunction. ALS, FTD or unspecified dementia was detected in 3 pedigrees in first-degree relatives of the SALS patients. Interestingly, cases carrying the *C9ORF72* hexanucleotide expansion had a more aggressive clinical course than cases who did not carry any mutation. Conclusions: We believe that ALS with *C9ORF72* hexanucleotide expansions, which probably represents a sizable proportion of apparent sporadic ALS in the Italian population [3], might be considered as the "trait d'union" between ALS and FTD and should be broadly investigated to clarify the pathological processes causing the spatial and temporal continuum or overlap between these disorders.

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Changes in the hippocampus of the R6/2 mouse model of Huntington's disease

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Introduction: Huntington's disease (HD) is an autosomal dominant neurodegenerative disease due to an expansion of a trinucleotide repeat in IT15 gene encoding for the protein huntingtin. Motor dysfunction, cognitive decline and psychiatric disorder are typical clinical signs of HD. The mutation leads to atrophy of the striatum and, later, of the cortex. However, the involvement of hippocampus in HD is gaining momentum. Indeed, cognitive signs are often the presentation of the disease. In HD, mutated huntingtin causes a major loss of brain derived neurotrophic factor (BDNF), causing striatal atrophy. In fact, BDNF, essential for the survival of the striatum, is transported anterogradely from the projecting cortical neurons. However, BDNF is also known to play a critical role in the synaptic plasticity underlying the acquisition and/or consolidation of certain forms of memory. We studied changes in hippocampal BDNF synthesis, and in CREB, and MAP kinases in the R6/2 mouse model of HD. Methods: PDEIV inhibitor rolipram or saline was administered to R6/2 or wild-type mice which were sacrificed at 5, 9, 13 weeks of age. In the hippocampus, pCREB, pERK, BDNF proteins were studied by means of immunofluorescence. Results: Our data show that BDNF is severely decreased in the hippocampus of R6/2 mice, while rolipram treatment restored physiological levels. Moreover, pCREB and pERK changes parallel those of BDNF. Conclusions: Our study demonstrates the involvement of hippocampus in the pathology of R6/2 model of HD, and correlates the beneficial effects of PDEIV inhibition with increased hippocampal levels of BDNF, pCREB and pERK.

Adult onset recessive Neuronal Ceroid Lipofuscinoses (NLCs) linked to progranulin mutations

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The NCLs are childhood and adult onset (ANCLs) progressive neurodegenerative diseases. Childhood forms are caused by mutations in at least ten genes, however, only more recently genetic mutations causing dominant and recessive ANCLs have been discovered. To identify the genetic cause of ANCL, in an Italian family, after excluding known genes, we performed hypothesis-free linkage analysis and exome sequencing in two affected siblings and their healthy parents. Patients were affected by an early onset (22 and 23 years) retinal dystrophy associated with cerebellar atrophy, seizures, ataxia and cognitive change. Electron microscopic (EM) examination of proband's skin biopsy showed fingerprint profiles (FFPs) in glandular cells and in endothelium. Unexpectedly, exome sequencing demonstrated an homozygous 4 bp deletion in the progranulin gene (*GRN*), resulting in a frameshift and premature termination

of translation residues downstream (p.Thr272SerfsX10). Heterozygous *GRN* mutations are a major cause of frontotemporal lobar degeneration (FTLD), and this specific variant is known to be one of the most common causes of FTLD in Italy; however, it has not previously been observed in the homozygous state. EM examination of brain tissue, from previously generated progranulin-deficient mice (*GRN*–/–), revealed abundant rectilinear and FFPs typical of NCL, thus endorsing the pathogeniticy of this homozygous mutation. Our findings reveal a link between NCLs and FTLD. The finding of similar and diagnostic EM features of NCL in human material and in progranulin deficient mouse supports the hypothesis that strikingly different phenotypes may be linked to this *GRN* mutation, depending on whether it is homozygous or heterozygous.

Aβ38 is abundant in the brain of patients with APP mutations inside the Aβ region and mainly builds up in vascular amyloid deposits

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Introduction: Aβ is the main component of amyloid deposits of Alzheimer disease (AD) and its aggregation into oligomers, protofibrils and fibrils is considered crucial in AD pathogenesis. Aβ42 is the major species in parenchymal plaques, while Aβ40 predominates in vascular deposits. The relevance of Aβ with other C-termini is still elusive. We have analyzed a shorter Aβ peptide, Aβ38, in the brains of patients with pathological conditions associated with Aβ deposition in the brain. Material and methods: Using an antibody specific for Aβ38, we performed immunohistochemical and immunofluorescence analysis of Aβ deposition in the frontal cortex of 12 patients with sporadic AD (sAD), 3 with hereditary cerebral hemorrhage with amyloidosis (HCHWA), 14 with familial AD (FAD) with APP and Presenilin1 or Presenilin2 mutations and 3 adult subjects with Down's syndrome. In addition, frozen samples were immunoprecipitated to separate Aβ38 oligomers by immunoblot. Results: A high percentage of Aβ38 positive deposits were found in patients with HCHWA and with FAD associated with APP mutations within the Aβ region, but not in patients with other APP mutations or genetic defects of presenilins. The positive patients showed also detectable Aβ38 oligomers in the cerebral cortex. Slight and uneven immunoreactivity, confined to vessel walls, was detected only in two sAD patients with severe cerebral amyloid angiopathy. Conclusion: Our results point to a relationship among Aβ38 cerebral deposition, the vascular and perivascular compartments and APP mutations within the Aβ region. This suggests that the molecular mechanisms of Aβ deposition in patients with these mutations may differ from those acting in other FAD and sAD.

Cutaneous sensory and autonomic denervation in Amyotrophic Lateral Sclerosis

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Introduction: Although motor impairment dominates the clinical picture of amyotrophic lateral sclerosis (ALS), sensory disturbances may occur along the course of the disease. Sensory symptoms and electrophysiological abnormalities have been reported in about 30% of ALS patients while sural nerve biopsy had revealed nerve abnormalities, with a prevalent involvement of large myelinated fibers in 91% of them [1]. These findings suggest that sensory involvement in ALS may be more than an occasional problem. Material and methods: To evaluate the involvement of peripheral sensory nerves in ALS, we extensively studied cutaneous innervation in 40 patients (20 male and 20 female age 64.3 ± 11.6 years) and 20 age and sex matched controls. Skin biopsies were taken from distal leg, thigh and fingertip using a 3 mm punch, after local injection of lidocaine. Samples were processed using indirect immunofluorescence techniques and an extensive panel of primary antibodies to mark both myelinated and unmyelinated somatic and autonomic nerve fibers. Quantification of epidermal nerve fibers (ENFs), Meissner corpuscles (MCs) and intrapapillary myelinated endings (IMEs) was performed using confocal images and dedicated software. Results: Compared with controls, ALS patients showed a loss of ENF in thigh ($p < 0.01$), leg ($p < 0.01$) and fingertip ($p < 0.01$) and a loss ($p < 0.01$) of Meissner corpuscles in glabrous skin. Epidermal denervation showed a length dependent pattern. In addition, a severe involvement of autonomic nerve fibers was present with a loss of sudomotor, pilomotor and vasomotor nerves. Noradrenergic pilomotor fibers appeared particularly affected. Conclusions: Our findings suggest that the involvement of last endings of sensory and autonomic nerve fibers is part of the neuropatological picture of ALS disease that should be considered a multisystemic degenerative disorder.

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Longitudinal magnetic resonance spectroscopy analysis in different transgenic mouse models of Alzheimer's disease

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Introduction: Understanding the metabolic changes induced in the brain by the Alzheimer's disease (AD) is an important task, since it would allow to identify clinical biomarkers suitable for

an early diagnosis. We present a magnetic resonance spectroscopy (MRS) study of the brain metabolism of three different mice models: single APP mutant, (PDAPP), double APPxPS-1 mutant (TASTPM), and triple APPxPS-2x-τ mutant (TAUPS2_APP). Methods: Mice were followed from 4 to 24 months. The ^{1}H spectra at short echo time were acquired with a PRESS sequence (TE = 10 ms) in two single voxels (4 and 7.5 mm^3) positioned respectively in the dorsal hippocampus and in the thalamus. The metabolite contents were expressed as ratios over Creatine (Cr). Results: A preliminary comparison showed that the models are characterized by different metabolic profiles of the hippocampus. Until 15 months a lower NAA/Cr (N-acetylaspartate) and a higher mIns/Cr (myo-Inositol) content were found only in the TASTPM. A significantly higher amount of τ/Cr (Taurine) was also estimated only in the PDAPP model. A progressive decrease of Glu/Cr (Glutamate) content was observed in the TASTPM mice, and, tentatively, in the PDAPP mice. Conclusions: These results indicates that the TASTPM model could better resemble the metabolic profile of the AD subjects. In particular the slow reduction of NAA/Cr and Glu/Cr can be attributed to neuronal loss and/or dysfunction. The research leading to these results was conducted as part of the PharmaCog consortium funded by the European Community's Seventh Framework Programme for the Innovative Medicine Initiative under Grant Agreement n°115009 (www.alzheimer-europe.org).

Mutations of FA2H and C19orf12 are rare in Italian patients affected by neurodegeneration with brain iron accumulation (NBIA)

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Introduction: Neurodegeneration with brain iron accumulation (NBIA) defines a wide spectrum of clinical entities characterized by iron accumulation in specific regions of the brain, predominantly in the basal ganglia. Material and methods: We evaluated the presence of *FA2H* and *C19orf12* mutations in a cohort of 46 Italian patients affected by early-onset NBIA, which were negative for the presence of mutations in *PANK2* and *PLA2G6* genes. Molecular genetics investigations and *in vitro* analysis were performed on available fibroblasts to verify the pathogenicity of the mutations. Results: We did not find any mutations in the *FA2H* gene, while we identified three patients carrying novel mutations in the *C19orf12* gene. Clinical presentations were quite heterogeneous and were characterized by a variable combination of dystonia, parkinsonism, spasticity and ataxia. Conclusions: The recent discovery of new genes responsible for NBIA extends the spectrum of the genetic investigation now available for these disorders and makes it possible to delineate a more clear clinical-genetics classification of the different forms of this syndrome. This study indicates that mutations of *FA2H* are absent in our cohort of patients, while mutations of *C19orf12* account for a minority of cases. Interestingly, a large fraction of patients still remains without a molecular genetics diagnosis, suggesting that additional NBIA genes are still to be discovered.

Modeling α-dystroglycanopathies in zebrafish

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Introduction: α-dystroglycanopathies are congenital muscular dystrophies (CMD) associated with hypoglycosylated α-dystroglycan (α-DG) in skeletal muscle. The clinical phenotypes include a striking range of clinical forms. At the most severe end of the clinical spectrum are conditions like Walker-Warburg syndrome (WWS), Muscle-Eye-Brain disease (MEB), and Fukuyama-type CMD. Individuals may also present in adult life with limb girdle muscular dystrophy and without associated brain or eye involvement. Mutations the three protein subunits of the dolichol-phosphate mannose synthase (DPM1-3) complex were also reported as additional etiologies in CMD, and an important link between muscular dystrophies and congenital disorders of glycosylation, though in yet unclear ways. *Danio rerio* (zebrafish) is being increasingly used for the functional genomics of neuromuscular disorders because of the several favorable characteristics of this model. Materials and methods: We cloned in silico the coding region of the three DPM genes by microinjection of specific amount of morpholinos into the yolk of one- to two-cell stage zebrafish embryos, we silenced the expression of *DPM1, DPM2* and *DPM3*. We analyzed gross anatomy at several embryonic stages and also mortality, muscular birefringence, and locomotion. Results: Zebrafish lacking *DPM2* or *DPM3* are characterized by ventral curvature, heart edema and, in some cases, heart abnormality. *DPM1* morphants showed a more severe phenotype with development delay and premature death. Zebrafish lacking *DPM2* or *DPM3* showed also a decreased muscular birefringence, indicating loss of the correct disposition of muscle fibers. The negativity to "touch response" of DPM morphants further enforce the presence of dystrophic phenotype. Conclusions: Zebrafish represents a suitable model organism for studying dystroglycanopathies and dissecting the pathogenesis of neuromuscular disorders.

AβPP and γ-secretase modulate τ-phosphorylation and cell cycle

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τ is a stabilizing protein detected in both cytoskeletal and nuclear cellular compartment of neuronal and non-neuronal cells, with a tightly

regulated phosphorylation, in physiology (mitosis for example – [1]) and pathology (neurofibrillary tangles – [2]). In Alzheimer's disease (AD) it is not yet established whether entangled τ represents a cause or a consequence of neurodegeneration. The leading hypothesis states that amyloid β-protein precursor (AβPP) plays a pivotal role in AD producing toxic amyloid-β peptides (Aβ), while hyperphosphorylated τ represents a secondary damaging events caused by Aβ [3]. However, here we provide evidence that AβPP, when overexpressed, modulates the phosphorylation of τ in mitotic and pathogenic phosphoepitopes during cell cycle [4]. We show that this event is strictly connected to its processing mediated by g-secretase and to its C-terminal consensus sequence YENPTY required for the interaction with Grb2. We also show that AbPP modulates the intracellular localization of phospho-τ, reducing the nuclear pool and the overall ratio nuclear/cytoskeletal [4]. We hypothesize that the AβPP-dependent phosphorylation of τ is involved in physiological processes such as chromosomal stability [5], cell motility and cell cycle progression, and that may lead to the formation of neurofibrillary tangles and to neurodegeneration when occurring in postmitotic neurons. Our hypothesis is that AβPP and presenilins, beside Ab formation, are involved in modulation of phosphorylation of τ and cell cycle dynamics.

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Biomarker for frontotemporal lobar degeneration (FTLD) with TDP pathology: the circulating lymphocytes

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Introduction: FTLD encompasses 3 groups based on the specific protein accumulated in neuronal cytoplasm and detected by immunohistochemistry: FTLD-τ, -TDP-43, -FUS. Biomarkers reflecting the specific neuropathological features could be crucial for the prediction of FTLD type during life and for future targeted treatments. In patients with amyotrophic lateral sclerosis (ALS), we showed that the abnormal cytoplasmic location of TDP-43 protein in lymphocytes mirrors the specific motor neuron pathology [1]. We therefore investigated whether the subcellular location of TDP-43 protein in lymphocytes of FTLD patients can point out the patients with FTDL-TDP, versus the other types of FTLD and other types of dementia. Material and methods: TDP-43 protein was extracted from circulating lymphocyte of 10 patients with FTD-FTLD; the subcellular distribution was analyzed by Western immunoblot and immunocytochemistry. Ten healthy subjects and 10 demented patients with non-FTD clinical diagnosis were the control groups. Results: In lymphocytes of 3 FTLD patients the TDP-43 protein was abnormally localized in the cytoplasm; in the other demented patients, and in healthy controls, TDP-43 was normally localized in the nucleus of lymphocytes. Conclusion: It is possible that the abnormal cytoplasmic localization of TDP-43 of lymphocytes found in a subgroup of FTD patients mirrors the neuronal pathology and, thus, be a marker of FTLD-TDP type. Only the neuropathological assessment will validate the diagnostic role of these results. Were this approach confirmed, lymphocytes could be informative of TDP-43 mislocalization in nervous tissue and biomarker of FTLD type in the individual patient.

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Effects of controlled delivery of FGF-2 on neural stem cell growth in vitro

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Neural stem cells (NSCs) are the self-renewing, multipotent cells that generate the main phenotypes of the nervous system. NSCs hold the promise for cell-based therapies in human neurodevelopmental and neurodegenerative diseases such as Alzheimer's and Parkinson's diseases. Unfortunately, several concerns with NSC cultures currently may limit their therapeutic promise. One major problem is the inability to precisely control stem cell behavior in culture, such as proliferation and specification into different cell types. NSCs are grown in serum-free media supplemented with several hormones and cytokines. It has been established that the adult mouse forebrain contains NSCs that can be cultivated in vitro when EGF or FGF-2 or their combination is provided. In particular, FGF-2 was shown to promote the growth rate of NSCs in vitro thereby maintaining their multilineage differentiation potential. However, FGF-2's susceptibility to enzymatic degradation may limit its clinical applications. We evaluated whether a device containing heparan sulfate (HS), which is a co-factor in growth factor-mediated cell proliferation and differentiation, could potentiate and prolong the delivery of fibroblast growth factor-2 (FGF-2) and thus

improve in vitro NSC cultivation. Our research showed that cultivation of NSCs in media with a controlled release of FGF-2 increased their proliferation rate, reduced the apoptosis and the senescence. In these experimental conditions NSCs preserve their stemness properties for a longer period of time compared with controls.

Efficient in vitro delivery of cationic nanoparticles loading siRNA targeting mutant huntingtin

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Introduction: Clinical applications of RNA-based therapeutics such as small interfering RNAs (siRNAs) have been limited mainly due to low intracellular delivery efficiency in vitro and in vivo. To enhance gene delivery effect, various cationic complexes have been developed for delivering plasmid DNA, antisense, or siRNA into cells. However, the use of cationic vectors for clinical applications has been severely limited by their high toxicity, low serum stability, nonspecific immune-stimulating effects, and poor biodegradability. In order to overcome these hurdles in gene therapy and improve gene delivery efficiency, we developed a copolymer composed of acetylated PEI (AcPEI) and poly(D,L-lactide-co-glycolide) (PLGA). The biodegradable PLGA-AcPEI nanoparticles were tested by loading the siRNA of mutant huntingtin in fibroblasts from control and heterozygotic HD patients [1]. Materials and methods: The PLGA-based nanoparticles presented in this study were synthesized by emulsion evaporation method, characterized by dynamic light scattering, and used as vector carrier for siRNA transfection in fibroblast cells. Quantitative PCR reactions were performed with primers to amplify mutant huntingtin or β-actin mRNA using the Quantitect SYBR Green PCR kit. Data were analyzed using the 2-ΔΔCT method [S3] and β-actin mRNA for normalization. Results: PLGA-AcPEI nanoparticles exhibited a higher transfection efficiency and lower cytotoxicity in fibroblasts from control and heterozygotic HD patients compared to the PEI/siRNA complex. Real-time PCR experiments showed decreased expression levels of mutant Htt and almost normal expression level of wild-type Htt. We achieved improved gene selectivity and allele selectivity of mutant *HTT* allele silencing. Conclusions: Targeted reduction of mutant *Huntingtin* mRNA is considered an ideal strategy for treating HD. Our results demonstrate that PLGA-AcPEI nanoparticles are promising non-viral vectors for gene delivery in neurodegerative disease.

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Differentiation of neuroblastoma cells: putative in vitro models to investigate childhood neurodegeneration

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Introduction: The study of neurodegenerative diseases is hampered by the lack of a reliable *in vitro* model of human neurons. SH-SY5Y is a human neuroblastoma cell line that retain the capability to differentiate into neuron-like cells under conditional media. Our goal was to set a reliable differentiation protocol to get an *in vitro* model of neuron-like cells suitable for studying human neurodegenerative disorders of childhood. Material and methods: The differentiation protocol consisted of two steps: a "pre-differentiation phase" in which cells were pre-stimulated with 10 μM Retinoic Acid (RA) and a "differentiation phase", in which specific media were used to induce neuron-like morphology. Different conditions (cellular density, media, FBS percentage and chemical factors concentrations) were tested during both phases. Cells were analyzed for the expression of neuronal markers (bIII tubulin, MAP2, neurofilament) by both biochemical and morphological tools. Results: Neurobasal medium enriched with specific factors (B27, rhBDNF, KCl, dbcAMP) was more effective to induce neuron-like morphology on SH-SY5Y than other media; cells showed extended neuritis and branches and the expression of neuronal marker increased. In addition, less apoptotic nuclei were seen in comparison with DMEM/F12-serum free medium added with RA and rhBDNF. Conclusions: This *in vitro* model could be an useful tool to investigate hereditary degenerative diseases affecting the nervous system. In particular, the combination of neuronal differentiation protocol on SH-SY5Y to obtain neuron-like cultures and the silencing approach to modulate the expression of disease-related genes will help us to shed light on pathogenetic mechanisms underlying neurodegenerative disorders of childhood.

Dysferlin expression in peripheral blood mononuclear cells (PBMC) correlates with that in skeletal muscles and supports the diagnosis of LGMD2B

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Introduction: Dysferlinopathies (LGMD 2B) are caused by mutations in the dysferlin gene (DYSF) and present with a wide phenotypic variability that includes distal forms, such as Miyoshi myopathy and a proximal form as limb girdle muscular dystrophy 2B (LGMD2B). The diagnosis is complex for clinical variability and the secondarily reduced dysferlin muscle expression due to primary defects in other genes. Dysferlin is also expressed in peripheral blood mononuclear cells (PBMC) and this property was used for not invasive diagnosis. Aim of the study was to determine whether dysferlin expression in PBMC cor-

relates with that in skeletal muscles. Patients and methods: Dysferlin expression in skeletal muscle and PBMC was studied by Western blot analysis in 6 patients with a diagnostic suspicion of LGMD2B, and in 3 samples from patients affected by LGMD2B molecularly confirmed, as positive controls. PBMC were isolated from whole blood by Ficoll-Hypaque gradient centrifugation technique, according to the manufacturer's instructions. WB was performed using a mouse monoclonal antibody to dysferlin (NCLHamlet, Novocastra, Newcastle, UK) and a mouse monoclonal antibody to actin (Sigma-Aldrich Quimica, Madrid, Spain) as a loading control. Dysferlin expression obtained on PBMC was compared with that obtained on muscles and with the results of molecular analysis. Results: A good correlation between skeletal muscle and monocyte dysferlin expression was found. A complete absence of dysferlin was always associated with homozygous mutations in the dysferlin gene, whilst reduced amounts of dysferlin were associated heterozygous mutations. Conclusions: The findings obtained using PBMC assay are fully consistent with the results from muscle immunodiagnosis. The new technique represents a reliable method to diagnose dysferlinopathies helpful in the case of lacking/ insufficient muscle samples or patient's refuse of a further biopsy, or to confirm a carrier status.

Hirayama disease: effect of neck flexion on somatosensory evoked potentials and Magnetic Resonance Imaging

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Introduction: Hirayama disease (HD) is a rare disorder characterized by juvenile onset of unilateral or asymmetric wasting and weakness of distal upper limb muscles, due to abnormal forward displacement of the cervical dural sac. We studied a series of 7 Italian HD patients with the aim of investigating electrophysiologically (somatosensory evoked potentials – SEPs) and neuroradiologically (spinal MRI) the dynamic changes of the cervical dural sac and spinal cord during neck flexion. Methods: HD diagnosis was made according to clinical criteria indicated by Chen et al. [1]. All patients underwent SEPs and spinal MRI in neutral and fully flexed neck position (not inferior to 35 degrees). Results: Seven HD patients from 6 families were recruited (6 males, 1 female; mean age at onset 19 years, range 15 – 32 years). Six patients had unilateral or asymmetric weakness and wasting of distal upper limbs, whereas 1 had predominant proximal involvement. No patient had sensory abnormalities at clinical examination. Spinal MRI revealed anterior displacement of the posterior dura of the cervico-thoracic spinal cord on neck flexion in 5 out of 7 patients. In 1 case detachment was anterior rather than posterior, whereas his father was the only case without dural abnormality. Four patients had cervical intramedullary signal abnormalities and 5 had focal cervical cord atrophy. All patients showed normal N13 potential at SEPs in neutral position, whereas in all patients neck flexion caused N13 amplitude reduction and/or latency prolongation (more evident on the clinically involved side). Conclusion: The role of SEPs in HD assessment is debated. Neck flexion produced significant changes in N13 potentials in all our HD patients whereas only 5 of 7 had MRI changes suggestive of HD. Therefore, SEPs proved more sensitive than MRI in diagnosing HD. Neck flexion corner not inferior to 35 degrees is probably a key factor in determining SEP sensitivity.

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Cognitive stimulation and ApoE genotype in non-demented elderly subjects: a randomized controlled study (RCT)

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Introduction: Dementia is the most troubling neurodegenerative syndrome. The increasing prevalence and incidence and the current lack of effective treatments provide a strong incentive for an improved therapeutic strategy. Epidemiological studies have proved the effectiveness of non-pharmacological interventions in preventing or delaying cognitive impairment in non-demented elderly subjects but little is known about the influence of APOE genotype on these treatments. The aim of this RCT was to test the effectiveness of cognitive stimulation in non-demented elderly subjects in relation to APOE genotype. Methods: 77 non-demented cognitively healthy subjects with familiarity for sporadic dementia (NDFAM) and 54 subjects with mild cognitive impairment (MCI) were separately randomly assigned to a "treatment" (ten sessions of cognitive stimulation) and a "control" group (two sanitary education sessions). A short neuropsychological test battery was blindly administered to all participants before and after the intervention. The APOE gene was typed by real-time allelic discrimination assay. Results: Performance in the neuropsychological tests after cognitive stimulation registered a greater improvement in NDFAM than in the MCI subjects, while a significant difference between the "treatment" and the "control" group was found only for NDFAM. Furthermore subjects carrying at least an APOE-ε4 allele, a risk factor for dementia, were less likely to benefit from the cognitive stimulation. Conclusions: Participating to the cognitive stimulation, compared with participating to sanitary education, resulted in greater improvement for NDFAM but not for MCI. Furthermore, the extent of the benefit arisen from cognitive stimulation was related to the presence of the risk factor APOE-ε4 allele.

Hypercholesterolemia is an important risk factor for mild cognitive impairment in APOE-ε4 carriers

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Introduction: Hypercholesterolemia is a questionable risk factor for dementia, but people with familial hypercholesterolemia (HC) have a higher incidence of mild cognitive impairment (MCI) compared with those without HC. In the present study we sought to investigate whether HC could be a risk factor for MCI elderly subjects by itself and in conjuction with the APOE-ε4 allele. Materials and methods: We typed APOE gene in 1,322 participants of the population-based Invece.Ab study (NIH-Clinical Trials. Gov: NCT01345110), a longitudinal study focused on all residents of Abbiategrasso (MI) born between 1935 – 1939. HC was registered in the anamnesis while MCI was diagnosed after the administration of a neuropsychological test battery and a medical evaluation. Results: More than 30% of the subjects involved in the present study were affected by HC. HC and non-HC subjects were equally distributed between MCI and cognitively healthy subjects (CT) (χ^2 = 0.00, p = 0.98). Furthermore, MCI subjects and CT had a similar distribution of the APOE-ε4 allele (χ^2 = 1.88, p = 0.17). Interestingly, APOE allele E4 had a highly different distribution between MCI and CT within the subgroup of subjects affected by HC (χ^2 = 6.46, p = 0.01). Conclusions: Our results suggest that HC is not a risk factor for MCI by itself but could be a powerfull risk factor in conjuction with at least one APOE-ε4 allele. This result is particularly strong (p = 0.01) and noteworthy because of the high prevalence of hypercholesterolemia in elderly subjects. This hypothesis will be tested longitudinally in the ongoing follow-up evaluation of Invece.Ab study.

Adiponectin reduces cell proliferation in glioblastoma cells through a prolonged activation of MAPK (Erk1/2)

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Adiponectin (Acrp30), is abundantly synthesized and secreted by fat cells and represents a key regulator of insulin sensitivity and inflammation [1]. Most of the biological effects of Acrp30 are mediated by AdipoR1 and AdipoR2 receptors, causing the activation of protein kinases, mainly AMP kinase and MAP kinases [2]. Recently, it was shown that decreased concentrations of Acrp30 are involved in development and progression of various types of malignancies [3]. At present there are no data on Acrp30 effect on glioblastoma (GBM) tumors. To investigate these aspects we analyzed human GBM tissues looking at the presence of Acrp30 receptors, and to investigate the effects of Acrp30 in GBM proliferation we used the human GBM cell lines U87-MG and U251. Here we show the expression of Acrp30 receptors – AdipoR1 and AdipoR2 – mRNAs in a high percentage of human glioblastoma (GBM) tissues, with a co-expression of both receptors in 7/12 (58.3%) tumors. We demonstrated that Acrp30 treatment induced a significant arrest in G1-phase of the cell cycle in U87 and U251 cell lines, resulting in a significant inhibition of DNA synthesis and cell proliferation rate. The reduced growth rate correlates with a prolonged activation of ERK1/2 and Akt kinases. Thus, our results suggest that Acrp30 may represent a novel endogenous regulator of GBM proliferation and, consequently, a novel pharmacological target for this still incurable neoplasia.

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Adult human fibroblasts express Notch3

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Introduction: Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a systemic arteriopathy with exclusively neurological symptoms. Clinical features consist in early onset strokes, migraine, vascular dementia, seizures, and psychiatric disorders. Degeneration of smooth muscle cells (SMCs) of tunica media and extracellular accumulation of granular osmiophilic materials (GOMs) are the hallmarks of this disease. Notch3 gene mutations have been associated to CADASIL occurrence. Studies evaluating Notch3 expression in human differentiated cells and adult tissues have shown high Notch3 levels only in vascular SMCs, but some evidences suggest that other cells could express Notch3. Moreover, as pericytes degeneration and GOMs accumulation near pericyte membrane were demonstrated in CADASIL, it was hypothesized that pericytes produce Notch3 and could contribute to the cerebrovascular dysfunction in CADASIL. Our aim was to evaluate the Notch3 expression in human fibroblasts derided by skin biopsies of normal subjects and CADASIL patients. Materials and methods: A 5 mm diameter skin patch was withdrawn and split into three portions, one for optical microscopy, a second for electron microscopy, and a third for fibroblasts colture. Notch3 expression was investigated by Western blotting, immunocytochemistry, and RT-PCR. Results: Notch3 expression

was demonstrated in fibroblasts by RT-PCR as well as Western blotting and immunocytochemistry. Moreover, we identified a new isoform of Nocth3 in fibroblasts. Conclusions: Notch3 expression is not restricted to vascular SMCs and other adult human mesodermal cells can express Notch3. These findings can be useful in understanding the role of pericytes and other vascular mesodermal cells in CADASIL pathogenesis.

Agar/Carbomer-based hydrogels as resveratrol delivery systems

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Introduction: Drug delivery systems based on injectable hydrogels represent an innovative approach for an effective treatment of many pathologies, such as neurodegenerative disorders. We aim to validate hydrogel-based systems loaded with resveratrol, a polyphenol with potential neuroprotective properties, also thanks to its activation of sirtuin 1 (Sirt1). They can be injected subcutaneously and, unlike oral administration, act as drug reservoirs, allowing a controlled, continuous and more efficient transdermal release profile. Materials and methods: Initial water content and swelling behavior with time in cell culture medium, phosphate buffered saline solution (PBS) and water at different pHs were evaluated for novel Agar/Carbomer (AC) and Agar/Carbomer/poly(ethylene glycol) (PEG) hydrogels. Their biocompatibility was tested with two cell lines: after incubation with hydrogel extracts L929 cell metabolic activity was quantified by Alamar Blue assay at different time points, while U-87MG cell viability was evaluated after extraction from matrices 3 days after embedding following trypan blue staining. Concerning resveratrol release profiles, preliminary investigations were performed spectrophotometrically. Results: The highest initial water contents (> 90%) have been obtained for AC hydrogels. Swelling ratio is lower at pH = 2 than at pH = 7 or 12, while it is higher in cell culture medium than in PBS. For all hydrogels, L929 metabolic activity increases with time, while U-87MG viability is greater for PEG-modified matrices. Investigations concerning resveratrol release profiles are ongoing. Conclusions: Results suggest tested hydrogels are interesting candidates as drug delivery systems. Examined chemical-physical properties vary with composition, but most suitable matrices will be identified focusing on resveratrol release profiles.

Postganglionic autonomic involvement in multiple system atrophy: a quantitative study of sudomotor innervations

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Introduction: Autonomic failure is one of the main manifestations of multiple system atrophy (MSA). Solid evidences point toward the impairment of preganglionic structures as its causative moment, however, an involvement of postganglionic fibers has been also suggested. Material and methods: We evaluated postganglionic involvement in MSA, quantifying sudomotor nerves in 3-mm punch skin biopsies from fingertip, thigh and leg of 17 patients (11 male and 6 female; age 59.1 ± 8.5) with a diagnosis of probable MSA. A group of 16 healthy subjects (7 male and 9 female; age 56.9 ± 7.4) was analyzed as control group. Skin samples were processed by indirect immunofluorescence to visualize sudomotor fibers using pan neuronal (PGP) and specific cholinergic (VIP) markers. Total length of sudomotor nerves was measured on digital confocal images using image analysis software (Autoneuron, MicroBrightfield). Results were compared to nerve density values obtained using an unbiased stereological method. Results: Measurements of sudomotor nerve length, favorably compared to the unbiased stereological evaluation. Total length of cholinergic sudomotor innervation per volume of glandular tissue (mm/mm^3) was reduced in patients in all the examined sites (0.104 mm/mm^3 vs. 0.191 mm/mm^3, $p < 0.01$, in fingertip; 0.074 mm/mm^3 vs. 0.172 mm/mm^3, $p < 0.01$, in thigh; 0.071 mm/mm^3 vs. 0.169 mm/mm^3, $p < 0.01$, in leg). The density of the total sweat gland innervation in patients compared to controls was 0.218 mm/mm^3 vs. 0.210 mm/mm^3 , $p > 0.05$,in fingertip; 0.147 mm/mm^3 vs. 0.198 mm/mm^3, $p = 0.05$, in thigh; 0.171 mm/mm^3 vs. 0.214 mm/mm^3, $p < 0.05$, in leg. Conclusions: We observed cholinergic sudomotor denervation in patients affected by MSA using a simple quantitative method that compared favorably with an unbiased stereological method used as gold standard. Our data support the hypothesis that a postganglionic impairment occurs in MSA and may be responsible, at least in part, of dysautonomia in these patients.

BPIFB4 missense variants associate with exceptional longevity in independent populations and influence cell signaling

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A recent Genome-Wide Association Study (GWAS) for exceptional longevity in Southern Italian Centenarians (SICs) identified six SNPs that were either non-synonymous or non-synonymous taggers with a $P < 1 \times 10^{-4}$. Their cross-validation in an independent population of 1628 German centenarians and 1104 controls have shown that rs2070325 replicates the previous association with SICs (OR: 1.42 and p = 0,0018 in Germans; OR: 2.42 and p = 0,000058 in SICs). Further analysis of rs2070325 in 1461 US centenarians and 526 controls confirmed the association (OR: 1.617 and p = 0,002). Rs2070325 is a frequent (35%) missense mutation (Ile268Val) in BPIFB4, a gene under "balancing" selection, and shows strong LD ($r2 = 0.93$ / $D' = 0.98$) with rs2889732 (Asn320Thr), rs11699009 (Phe527Leu) and rs11696307 (Thr533Ile) ($r2 > 0.83$ / $D' > 0.95$). The first two

amino acid changes (DM) reside in a 200 amino acid stretch that is under "purifying" selection. BPIFB4 is a secreted protein that belongs to a large family of BPI/LBP/Plunc-like proteins characterized by lipid-binding pocket (LBP). RT-PCR detected BPIFB4 in immortalized, dedifferentiated, undifferentiated, and stressed cells. Furthermore, HEK293T cells transfected with wild type (WT) and/or DM induced dramatic changes in PKC alpka, GSK3 beta, NFKB and MAPK signals. Cell medium analysis showed that HEK293T transfected with DM protein improved secretion of BPIFB4 together with increased secretion of 14-3-3. Furthermore, S phase is increased in BPIFB4 transfection. Being that the signals modulated by BPIFB4 are important for the cell death/cell survival decision, further studies are needed to understand why these mutations are protective for human health, as indicated by their enrichment in the genome of long-lived individuals (LLI).

Variable protease-sensitive prionopathy and Gerstmann-Sträussler-Scheinker disease share many major clinico-pathological and biomolecular features

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Variable protease-sensitive prionopathy (VPSPr), a recently identified prion disease, displays features that are reminiscent of Gerstmann-Sträussler-Scheinker disease (GSS) while it differs from other prion diseases. Specifically, in VPSPr, like in many subtypes of GSS, the abnormal PrP forms a "ladder-like" pattern on Western blot (WB) and amyloid plaques are often present in the cerebellar cortex. However, unlike GSS, no PrP gene mutation has been found in VPSPr. Another puzzling similarity between GSS and VPSPr is the presence of τ-pathology which, however, has distinct topographies in the two conditions. The τ-pathology associated with the presence of abnormal hyper-phosphorylated τ is also present in other neurodegenerative diseases such as Alzheimer's disease (AD), Pick disease (PiD) and frontotemporal dementia (FTD). Four distinct types of disease-associated τ (τ^{Dis}) have been identified. Here we characterize by immunohistochemistry and WB the abnormal τ associated with VPSPr and compare it with that of GSS subtypes, FTD, PiD and AD. The similarities with GSS along with the lack of PrP gene mutations raise the question as of whether VPSPr should be considered the long sought sporadic form of GSS. (Supported by CJD Foundation Grant 2009; NIA AG-14359, NINDS NS052319, NIH NS 062787, CDC UR8/CCU515004 and Charles S. Britton Fund).

No heart involvement in SBMA patients

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Introduction: Spinal and bulbar muscular atrophy (SBMA) is an adult-onset, X-linked, lower motor neuron disease, characterized by slowly progressive muscle weakness and atrophy. The disease is caused by an expansion of a CAG repeat encoding a polyglutamine tract within the androgen receptor (AR) gene. Nuclear accumulation of pathological AR, which is toxic to motor neurons, has been observed in tissues other than the nervous system including the heart. Materials and methods: To test the hypothesis of the presence of heart disease in SBMA we carried out a full cardiologic evaluation (12-lead ECG, Echocardiography and 24-h ECG Holter) in 26 genetically defined SBMA patients. Results: Patients' age range was 32 – 75 years (mean age 54.4 years). Ten patients had high blood pressure and were under antihypertensive medications. No patients displayed clinical signs of heart disorders at the cardiologic examination. The 12-lead ECG findings were normal or consistent with left ventricle (LV) hypertrophy in the oldest patients suffering form high blood pressure. Similarly, echocardiography showed no abnormalities other than mild concentric LV hypertrophy in patients with hypertension. No patients showed significant rhythm abnormalities at the 24-h ECG Holter. Conclusions: Our findings do not support the hypothesis of a primary heart involvement in SBMA.

Ectopic ATP production in myelin sheath: correlation between demyelination and axonal degeneration in multiple sclerosis

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Introduction: In Multiple Sclerosis (MS), the myelin loss is associated with an axonal degeneration, the major determinant of the neurological disability. It was hypothesized that axonal degeneration could depend on an energy imbalance consequently to a lack of trophic support by myelin [1]. Recently, we have reported that myelin is a site of oxidative phosphorilation (OXPHOS), producing ATP by oxygen consumption [2, 3]. To understand the "energetic role" of myelin in MS, the OXPHOS functionality was studied in isolated myelin vesicles from MS plaques at different activity degrees, comparing with healthy samples. Moreover, to investigated the production of free radicals by myelin, the presence of lipid peroxidation and the detoxificant enzymes were investigated. Material and methods: Confocal laser microscopy as well as oxymetric, fluorimetric, luminometric, spectrophotometric and semiquantitative Western blotting analyses were performed. Results: In myelin from MS plaques, the confocal signal of MBP and complex IV colocalized and decreased pararelly. Oxygen consumption, activity of the four respiration complexes and ATP synthesis were impaired with respect to the healthy controls, even though the expression of the OXPHOS proteins was the same in all samples. These decreases were directly proportional to the activity of MS plaques. Moreover,

a production of free radical was observed in IMV from MS. Conclusions: Data demonstrated that the OXPHOS activity was compromised in damaged myelin from MS plaque, suggesting that the energetic support by myelin may be impaired. Moreover, we presume that this respiratory chain dysfunction could depend by the production of free radicals that, damaging the membrane, could cause further loss of myelin sheath. These data could give a new input to understand the correlation between the myelin loss and the axonal degeneration, clarifying the neuro-trophic role of myelin.

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Tankyrase inhibition in medulloblastoma cells by XAV939

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Introduction: Tankyrase (TNKS) is a member of PARP family involved in NHEJ stabilization, DNA-dependent protein kinase (DNA-PK); TNKS depletion results in degradation of catalytic subunit of DNA-PK (DNA-PKcs) causing increased sensitivity to ionizing radiation (IR). TNKS interacts with axin and stimulates its degradation arising in WNT pathway inhibition. Alterations in this pathway often occur in medulloblastoma (MB): post-operative radiotherapy is very effective for MB, but survivors have severe long-terms side effects and sometimes radiotherapy response is limited by intrinsic radioresistance. We showed the consequences of pharmacological TNKS inhibition on MB cells using XAV939, a novel small molecule targeting TNKS-PARP activity. Methods: ONS-76 MB cells were treated with XAV939 and/or IR (γ-ray, dose 2 Gy): MTT, growth-curve, trypan blue exclusion and adherent colony formation assays were performed. Axin, β-catenin and DNA-PKcs expression in response to XAV939 were evaluated by WB. Results: XAV939 treatment on MB cells resulted in a consistent decrease of the clonogenic and proliferative capability, not related to a cell mortality increase, showing its low toxicity. The co-administration of XAV939 and IR induced a further inhibition of cell proliferation and colony formation. We did not observe a statistically significant increase in cell mortality on XAV939 and IR treated cells compared to controls. After a second IR dose (2 Gy), in XAV939 treated cells mortality was double compared with controls. Conclusions: Our *in vitro* data showed that XAV939-mediated TNKS inhibition correlates both to cell proliferation decrease and to radio-sensitivity increase, suggesting TNKS as a possible therapeutic target to improve MB therapy.

The diagnostic challenge of the amyloid myopathy: a case report

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Introduction: Amyloid myopathy is a rare manifestation of primary systemic amyloidosis, presenting with proximal muscle weakness and increased creatine kinase level. We report a case of primary systemic amyloidosis with severe and rapidly progressive myopathy as the initial symptom. Case description: A 78-year-old woman came to our attention for a rapidly progressive proximal muscle weakness, with impossibility in rising the arms over 40°, in climbing the stairs or set up from a chair without support. No muscle atrophy was established and sensory functions were intact. Creatine kinase level resulted slightly increased. Electromyography showed a myopathic pattern; sensitive and motor nerves conductions were normal. The muscle biopsy revealed moderate myopathic changes, with scattered atrophic fibers, fibers degeneration and focal endomysial inflammatory infiltrate. The provisional diagnosis at this stage was considered to be an inflammatory myopathy. Immunoglobulin 0.4 mg/kg i.v. was given for 8 days, leading to a minor improvement in muscle strength, so that the treatment was repeated 1 times after 3 months. Nevertheless, the muscle weakness progressed within 2 months, however, forcing the patient to walk with bilateral support. The patient also began to complain heart failure symptoms due to left ventricular diastolic dysfunction. The cardiac magnetic resonance revealed a fibrosis pattern suggestive of amyloid cardiomyopathy. This diagnosis was proved by the subcutaneous adipose tissue aspiration resulting positive for Congo red staining. The muscle biopsy was then re-examined, and Congo red staining showed vascular and interstitial amyloid accumulation, compatible with the diagnosis of amyloid myopathy. The subsequent hematological evaluation confirmed that the patient was affected by primary systemic amyloidosis. Discussion: Although amyloid myopathy is a well described disorder, it is often overlooked because it is rarely observed in clinical practice and a broad span of differential diagnoses is needed. It is usually misdiagnosed as inflammatory myopathy.

Cell free microRNA and multiple sclerosis: possible promising biomarkers?

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Introduction: Emerging evidence underlines the importance of micro(mi)RNAs in the pathogenesis of multiple sclerosis (MS). The main aim of this study was to identify a specific profile of extracellular miRNAs in serum from MS patients

compared to controls. Material and methods: SAbiosciences miRNA PCR array containing 88 common miRNAs was used to screen miRNA levels in serum from 4 patients with relapsing remitting (RR)-MS, 4 with primary progressive (PP)-MS and 4 age matched controls. Best hits were first validated by real time PCR in an Italian cohort consisting of 15 serum samples from MS patients and controls, each and subsequently replicated in an American population of 30 MS patients and 30 controls. Results: The initial screening showed a generalized down-regulation of miRNA levels in patients respect to controls. Statistically significant decreased levels of miR-23a, miR-15b and miR-223 were observed in MS patients (–2.0-fold regulation, –2.9-fold regulation and –3.9-fold regulation respectively, $p < 0.05$). Results were then validated and replicated and the downregulation of miR-15b and miR-223 was confirmed ($p < 0.05$). Conclusions: This is the first attempt to determine circulating miRNAs in MS. miR-223 and miR-15 were already found to be dysregulated in cells from RRMS patients, highlighting a role of these miRNAs in MS. Moreover, the generalized trend towards miRNA down-regulation could determine an over-expression of target genes likely involved in the disease pathogenesis. Circulating miRNAs profiling could thus represent a new challenge in the research of easy detectable biomarkers of disease.

Sirtuins modulation and neurodegeneration

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Introduction: Human sirtuins (SIRT) are a family of seven conserved protein with NAD+-dependent deacetylase activity involved in gene silencing, cell cycle control, apoptosis and energy homeostasis. SIRT1 and SIRT2 may regulate aging and neurodegenerative disorders like Alzheimer's and Parkinson's diseases (AD/PD). SIRT1 is involved in the activaction of protective cellular pathways (antioxidant response, autophagy) while SIRT2 point out an opposite direction (neurotoxicity). SIRT3 was identified as a stress responsive deacetylase recently shown to play a role in protecting cells under stress conditions. We started to investigate SIRT (1-3) overexpression or modulation by small molecules in *in vitro* oxidative stress models. Materials and methods: To assess the effects of SIRT (1-3) on cell viability and oxidative stress response and to verify whether modulation of SIRT (1-3) by small molecules is beneficial we have developed human cellular lines overexpressing SIRT (1-3) (SHSY5Y and H4) and pathological *in vitro* models of AD/PD (amyloid-β-protein and DJ1M26I). Results: Our models confirm that SIRT1 has a protective role against oxidative stress. SIRT2 inhibition by the competitive inhibitor AGK2 enhances cell surviving in mutated DJ1 overexpression model. Data on SIRT3 suggest a prosurvival effect. In order to modulate SIRT3 activity we have tested selective activators and inhibitors. Conclusions: Our data suggest that the identification of small-molecule pharmacological modulators of sirtuins activity plays a key role as therapeutic tool to treat neurodegenerative disorders.

VEGF-A isoforms in human astrocytomas with different grade of malignancy

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Introduction: Gliomas are among the most vascularized human tumors, and disproportionate vasculature is induced by several pro-angiogenic factors produced by glioma cells. It has been demonstrated that caotic angiogenesis is one of the main feature of cancer. Vascular endothelial growth factor (VEGF-Aa, VEGF-Ab), a diffusible glycoprotein, is a widely over-expressed pro-angiogenic factor in most solid cancers and plays a critical role in various steps involved in angiogenesis including endothelial cell proliferation, migration and tube formation. The present study was aimed to analyze VEGF-A isoforms expression and its correlation with tumor vasculature in human astrocytomas with different grade of malignancy: low-grade astrocytomas (LGA), anaplastic astrocytoma (AA) and glioblastoma multiforme (GBM). As it is hypothesized that tumors with relatively higher pro-angiogenic (VEGF-Aa) than anti-angiogenic (VEGF-Ab) isoform expression will be more sensitive to anti-angiogenic therapy, because VEGF-Ab inhibits the effect of anti-VEGF-Aa antibodies, like bevacizumab, we secondly aimed to determine the VEGF-Aa/VEGF-Ab ratio. Material and methods: VEGF-A isoforms have been analyzed in tumor samples of 30 human astrocytomas (10 LGA, 10 AA and 10 GBM), using Real Time RT-PCR. Microvessel density, vessel maturity, and endothelial and tumor cell turnover with CD34, collagen IV, smooth muscle actin, Ki67/CD34 and caspase3/CD34 respectively performed by Western blotting. The angiopoietin-1/-2 balance, as an indicator of vessel stability, was assessed using real time PCR. Results: We found an over-expression of VEGF in all specimens. Furthermore, the level of expression increased proportionally to the histological grade of malignancy of astrocytic tumors. Also, we recorded a different expression of VEGF-Aa and VEGF-Ab isoforms in tumor tissue. Conclusions: Our preliminary findings may add a new information about the expression of angiogenetic factors, their ability to change tumor vasculature and their role in the response to antiangiogenic therapies.

Lewy body pathology and typical Parkinson disease in a patient with a (R275W) heterozygous PARK2 mutation

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Introduction: The role of heterozygous mutations of the parkin gene *(PARK2)* in the pathogenesis of Parkinson's disease is far from clear. We

report a patient with a heterozygous *PARK2* mutation (R275W, exon 7), clinical features of typical Parkinson's disease, neuropathological picture of diffuse Lewy body disease and a positive family history of Early-Onset Parkinson disease with two affected offspring. Case report: An 80-year-old male patient came to autopsy after an 18-year history of typical Parkinson's disease, characterized by asymmetrical onset of resting tremor, rigidity and bradykinesia, good response to levodopa, and late-onset dementia. Microscopic examination revealed severe neuronal depletion in the substantia nigra and locus coeruleus, where numerous α-synuclein-immunoreactive Lewy bodies and neurites were present. Lewy bodies were also present in lower brainstem regions (pons and medulla) and at the cortical level, both in limbic and neocortical regions. There was intense, widespread cortical deposition of β-amyloid, mostly in the form of diffuse plaques, involving also the striatum. τ-immunohistochemistry disclosed intraneuronal deposits (pre-tangles) at the level of the subiculum, transentorhinal and entorhinal cortex. Conclusions: To our knowledge, this is the first report of diffuse Lewy body pathology associated with a clinical history of typical Parkinson's disease in a patient with a heterozygous *PARK2* mutation. This suggests a possible role of heterozygous *PARK2* mutations in the pathogenesis of α-synucleinopathies.

Muscular dystrophy: Histological and immunohistochemical study on 226 cases

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Introduction: Muscular dystrophies are hereditary degenerative diseases of skeletal muscles. Their diagnosis is confirmed by histological and immunohistochemical examination of muscle biopsies, and genetic analysis. The aim of our study is to determine the epidemiological profile of muscular dystrophy in our population. Materials and methods: This is a retrospective study of 226 cases of muscular dystrophy, collected at the Laboratory of Pathology of the Hospital of Specialties in Rabat over a period of 12 years (1999 – 2010). We studied surgical muscle biopsies by using standard staining (HE, trichrome, PAS), methods of histo-enzymology (SDH, NADH). The immunohistochemical examination was performed in all cases, using anti-dystrophin 1, 2 and 3, anti-sarcoglycan α, β, γ, δ and anti-merosin. The anti-dysferlin antibody was used from 2009. Results: Our series was composed of 144 male and 82 female patients with a mean age of 28.07 years. On the histological and immunohistochemical study, we found 38.05% of dystrophinopathies; 27.87% of sarcoglycanopathies, essentially α-type (24.77%), 3.53% of dysferlinopathy, 3.09% of congenital muscular dystrophy due to deficiency of merosin. In 26.59% of cases, no protein deficiency has been identified. Conclusions: The immunohistochemical panel that we have allowed us to classify muscular dystrophies in 72.56% of cases. The design of new antibody market and the development of molecular biology will broaden the scope of these diagnostic conditions.

Ultrastructural pathology and molecular findings in 5 patients with adult Neuronal Ceroid Lipofuscinoses

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Introduction: The adult form of Neuronal Ceroid Lipofuscinoses (ANCL) is part of a heterogeneous group of progressive, inherited neurodegenerative diseases that occur mostly in childhood; two forms are known, according to the presence (ANCL-A) or absence (ANCL-B) of myoclonic epilepsy. Ultrastructural analyses reveal lipopigment and cytosomes in extra-cerebral neuronal tissues, or even in non neural cells. ANCL-A is associated with mutations in *CLN6*, a gene coding for a trans-membrane protein of the endoplasmic reticulum (ER); the rare autosomal dominant (AD) form is linked to mutations in *DNAJC5*, which encodes a cisteine-string protein alpha. No ANCL-B gene has been identified so far. Materials and methods: Five ANCL patients underwent ultrastructural investigations of extra-cerebral tissues (rectal biopsy, skin, skeletal muscle, blood lymphocytes) and genetic analyses. Tissue samples underwent current EM procedures. Analysis of *CLN6* and *DNAJC5* was based on standard PCR methodologies and capillary Sanger sequencing. Results: Three ANCL-A patients carried mutations in *CLN6* (two cases) and in *DNAJC5* (one case); no mutations were detected in both ANCL-B patients. Lipopigment material was the most common storage in any examined tissue. FPPs were detected in neuronal cells in patients harboring mutations in *CLN6*, whereas GRODs were observed in the patient mutated in *DNAJC5*. Conclusions: Increased amount of lysosomal storage was shown in all tissues of the ANCL patients, whereas cytosomes were mainly detected in ganglionic neurons. Rectal biopsy can lead to the appropriate molecular genetic investigations for suspected ANCL-A cases. A combined ultrastructural-molecular approach is recommended in the diagnostic evaluation of ANCL patients.

In vitro studies suggest reduced expression of methyl cytosine protein binding 2 (MECP2) affects cell commitment and maintenance in neurons by triggering senescence, new perspective for Rett syndrome

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Introduction: Methyl cytosine protein binding 2 (MECP2) binds preferentially to methylated CpGs and regulates gene expression by causing changes in chromatin structure. The mechanism by which im-

paired MECP2 activity can induce pathological abnormalities in the nervous system of patients with Rett syndrome (RTT) remains unknown. Studies in different animal models have produced conflicting results. In a mouse model of RTT syndrome, the results indicate that MECP2 is involved in the maturation and maintenance of neurons, whereas in *Xenopus* embryos, MECP2 mutations seem to affect neural cell fate decisions. Material and methods: To gain further insight into the role of MECP2 in human neurogenesis, we compared the neural differentiation process in mesenchymal stem cells (MSCs) obtained from a RTT patient and from healthy donors. We further analyzed neural differentiation in a human neuroblastoma cell line carrying a partially silenced *MECP2* gene. Results: Senescence and reduced expression of neural markers were observed in proliferating and differentiating MSCs from the RTT patient, which suggests that impaired activity of MECP2 protein may impair neural differentiation, as observed in RTT patients. Next, we used an inducible expression system to silence *MECP2* in neuroblastoma cells before and after the induction of neural differentiation via retinoic acid treatment. This approach was used to test whether MECP2 inactivation affected the cell fate of neural progenitors and/or neuronal differentiation and maintenance. Conclusion: Overall, our data suggest that neural cell fate and neuronal maintenance may be perturbed by senescence triggered by impaired MECP2 activity either before or after neural differentiation.

Mutation in the ferritin light chain gene in an Italian patient: clinical report and pathogenetic insight

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Introduction: Hereditary ferritinopathy (HF) is a rare genetic condition with autosomal dominant transmission caused by mutation in the ferritin light chain (*FTL*) gene. Ferritin is the main intracellular iron storage protein, having a central role in the regulation of iron metabolism. Neuropathologically, HF is characterized by abnormal iron accumulation in the basal ganglia and the presence of ferritin inclusion bodies (IBs) in neurons and glia. Defect of mitochondrial metabolism has also been reported. Progressive iron accumulation in the basal ganglia and other regions of the brain clinically results in extrapyramidal dysfunction. Materials and methods: We identified an Italian HF patient carrying the *FTL* c.469- 484dup16 mutation already described in Japanese patients. Results: The patient, a 41-year-old man, presented at latest examination with marked rigidity, ataxia and sporadic dystonic movements. Clinical symptoms started in early adulthood with tremor and mood disturbances. Standard magnetic resonance (MRI) of brain demonstrated typical alterations in basal ganglia and diffuses cortical atrophy. Serum ferritin levels were far below the normal range. A muscle biopsy showed normal activities of the respiratory chain complexes. Conclusions: Our data add to the heterogeneity of HF syndromes.

Autosomal dominant Alzheimer's disease with early frontal lobe involvement associated with the Met239Ile mutation of Presenilin 2 gene

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Introduction: Mutations in the *Presenilin 2* gene (*PSEN2*) represent the less frequent genetic cause of familial Alzheimer's disease (FAD). Only eight *PSEN2* mutations, reported in approximately twenty-seven families, satisfied strict criteria of pathogenicity. Case report: We reported a patient with early-onset FAD and the PSEN2 p.Met239Ile mutation, presenting with severe executive dysfunction and myoclonic tremor, associated with memory loss. Brain SPECT study showed an early hypoperfusion of the frontal cortex. Conclusions: We confirmed the pathogenicity of PSEN2 p.Met239Ile mutation and its heterogeneous phenotypic expression. The modulating effect of the *ApolipoproteinE* and *Prion Protein* gene polymorphisms on the phenotypic variability was not confirmed.

A novel PSEN1 mutation in a patient with early-onset Alzheimer's disease and prominent cerebellar ataxia

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Introduction: Familial Alzheimer's disease with mutations of *Presenilin 1* gene (*PSEN1*) manifests usually with early (< 65 years) and progressive impairment of episodic memory. Heterogeneity of clinical presentations was observed with myoclonus and seizures, spastic paraparesis, and extrapyramidal signs as early and prominent syndromes; exceptional cases had cerebellar ataxia as a heralding and dominating sign. Case report: A 32-year-old woman without family history for neurological disorders manifested a progressive impairment of short-term memory and attention with compulsive behavioral changes. By age 35, nocturnal myoclonus of limbs and severe cerebellar ataxia developed with gait unsteadiness, limb dysmetria and dysmetria; neuropsychological evaluation disclosed an impairment of verbal memory, a mild disexecutive syndrome and prosopoagnosia. At the age of 38 years, examination reveals a cerebellar-pyramidal syndrome with cognitive decline. Extensive laboratory investigations, performed to rule out an acquired or genetic ataxic syndrome, included molecular testing of SCA1,2,3,6, 17 and FRDA loci, as well as a Filippin test. MRI showed bilateral white-matter hyperintensities, enlargement of the subarachnoid spaces and slight cortical cerebellar atrophy. PET disclosed bilateral hypometabolism of the superior prefrontal, superior parietal and precuneal cortexes and, to a lesser extent, of the right anterior temporal cortex. Molecular analysis finally detected a novel non-con-

servative p.Thr147Pro mutation of PSEN1. A different p.Thr147Ile change was previously associated to typical FAD. Conclusions: The report emphasizes the clinical variability of PSEN1-associated FAD which may evolve with a prominent cerebellar syndrome.

Biocompatibility of a novel collagen/poly(ethylene glycol)-based hydrogel for protein delivery

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Introduction: Recombinant proteins such as Tat-HSP70 represent possible therapeutic agents to counteract dopaminergic dysfunction in Parkinson's disease. Their systemic administration shows some drawbacks, which may be overcome by developing a controlled release system [1, 2]. Aiming at this application, a biodegradable hydrogel was proposed and its biocompatibility investigated. Materials and methods: A novel collagen/poly(ethylene glycol)-based hydrogel was developed. Its initial water content was evaluated, swelling behavior followed with time after dipping in cell culture medium and morphology observed by a scanning electron microscope. Hydrogel biocompatibility was assessed in CD-1 mice: 48 h after subcutaneous injection cell density was evaluated in the exudates following trypan blue staining, while 7 days after injection in the *striatum* brain sections were stained by Nissl and Fluoro-Jade. Results: Hydrogels are semi-interpenetrating polymer networks with fibrillar but porous structures. Average initial water content is 98.6% and the highest swelling ratios have been obtained within the first 5 minutes. Cell density in the exudates is comparable between hydrogels and saline. Nissl has evidenced hydrogel presence does not modify brain cytoarchitecture, while Fluoro-Jade suggested neurodegeneration is mostly due to the needle insertion. Conclusions: Results suggest hydrogels elicit minimal inflammatory response and they pave the way for their exploitation in brain applications. Future investigations will focus on Tat-HSP70 release from proposed hydrogels.

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From conformational alteration to amyloidogenicity of 3 transthyretin (TTR) mutants

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Introduction: Familial amyloidotic polyneuropathies (FAP) are autosomal dominant systemic diseases characterized by amyloid deposition in peripheral nervous system. More than 100 different mutations of transthyretin (TTR) gene have been associated to FAP. TTR is a homotetrameric protein and carries retinol and thyroid hormones in blood and cerebrospinal fluid. In TTR amyloidosis, the amyloidogenic mutations reduce the stability of TTR tetramer and the partial denaturation of tetrameric TTR to monomer is followed by the formation of soluble oligomeric species and amyloid fibrils. The aim of this study is to investigate the relation between conformational alterations, tetramer stability, amyloidogenicity and toxicity by three recombinant TTR mutants. Materials and methods: The three mutants (V14N, V30M and L55S) and wild-type (WT) TTR were generated by plasmidic expression system, purified by nickel resin and verified by WIB. Quaternary structure was studied by gel filtration chromatography and thermodynamic analysis. Spectrophotometric techniques, electron microscopy and atomic force microscopy methods were used to investigate the formation of fibrillar and no-fibrillar TTR aggregates. Cell toxicity was evaluated on human neuroblastoma cell line IMR-32. Results: Comparing to WT, the three mutants were less able to maintain tetrameric conformation, less influenced by the T4 stabilizing effect on tetramer, more effective in amyloid formation, and more toxic. Among the three mutants, V14N and L55S produced more instable tetramers and were more able to from TTR aggregates and more toxic. Conclusions: Amyloidogenicity and toxicity of our mutants closely related to the stability of the protein.

Prognostic markers related to angiogenesis on pediatric ependymomas and medulloblastomas: study of 61 cases

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Introduction: Brain tumors are the major cause of cancer related mortality in children. Medulloblastoma and ependymoma represent 10% and 20%. Aim of our study was to characterize some neoangiogenesis related biomarkers in pediatric brain tumors, investigating potential prognostic and prospective therapeutic targets. Vascular endothelial growth factor (VEGF) promotes angiogenesis. Plasmalemma-vescicle associated protein (PV-1) and Caveolin (Cav-1) are localized in caveole of fenestrated endothelium with a structural role in permeability. Endoglin is a marker of activated endothelial cells within the tumor. Platelet-derived growth factor receptor (PDGFR) is implicated in tumorigenesis and angiogenesis. WT1 is a transcription factor involved on angiogenesis. Materials and methods: We performed an immunohistochemical study on 61 pediatric patients, which underwent surgery in our Institute. They were: 35 ependymomas and 26 medulloblastomas. We analyzed the

expression of: PV-1, Cav-1, endoglin (CD105), VEGF, PDGFR-A and WT-1. On some cases we confirmed the presence of mRNA of these molecules using Real Time-PCR. Results: We found PV-1, Cav-1 and endoglin expressed in neoplastic endothelial cells. We observed a significant different expression of WT1 in endothelial and neoplastic cells, related to malignancy grade, particularly in ependymomas. Co-expression of WT1 and VEGF was also detected in ependimoma and medulloblastoma. PDGFR-A is expressed in endothelial cells, related with cancer malignity. Conclusions: In our study Caveolin and WT-1 seem to be prognostic factors in ependymomas. The different expression profiles of these markers can help to evaluate the mechanisms of angiogenesis and of vasogenic edema, suggesting potential markers for appropriate selection of patients for anti-angiogenic therapies.

Metformin selectively inhibits the proliferative potential of tumor-initiating cells derived from human glioblastoma

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Introduction: Glioblastoma (GBM) cancer stem cells (CSCs) show neural stem markers, self-renewal, and high tumorigenicity (tumor-initiating stem cells, TICs) compared to the bulk of differentiated tumor cells. Clinically, drug resistance is the most important feature of TICs, likely playing a major role in GBM recurrence. The antidiabetic drug metformin is emerging as a potential agent selectively targeting CSCs. Methods: Culture and differentiation protocols were optimized for TIC cultures; MTT assay, FACS and confocal immunofluorescence were used for proliferation and characterization analyses; tumorigenic potential was assayed by mouse orthotopic xenografts. Results: Four cultures (TIC1-4) isolated from GBM post-surgical specimens were grown as neurospheres in a stem-permissive medium, fully characterized and monitored for marker expression (CD133, nestin), self-renewal, differentiation and tumorigenic potential. Metformin treatment significantly decreased sphere formation and TIC proliferation, through the inhibition of the Akt survival-pathway. The mean IC_{50} values in the 48-h viability assays were in the micromolar range (8.5 – 18 mM) for all the cultures and growth inhibitory effects were mainly cytostatic up to the IC_{50}, while differentiated TICs and normal mesenchymal stem cells, used as control, showed lower sensitivity. Metformin antimitogenic effects selectively act on CD133/nestin-positive cells, by reducing their proliferation index and rate of cell division in a dose- and time-dependent manner. Conclusions: These results suggest that the metformin-induced inhibition of cell division is an important mechanism in the control of GBM TICs, suppressing the proliferative advantage present in these cells. Thus, metformin could be useful for overcoming GBM therapeutic resistance. (Supported by AIRC grant #IG9089).

Addendum*

Decreased concentration of total Adiponectin, its HMW oligomers and mannose binding lectin in patients affected by Myotonic Dystrophy Type 1

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Introduction: Myotonic dystrophy type 1 (DM1) is a genetic disease characterized by muscular atrophy, by multisystemic clinical features affecting heart and the nervous and endocrine systems. DM1 complications include metabolic disorders and severe insulin-resistance. Adiponectin (Acrp30) is produced by adipose tissue and secreted in large amounts in the serum with beneficial effects on different metabolic alterations, first of all on insulin resistance. Serum Acrp30 is present as oligomers of low, medium and high molecular weight (LMW, MMW and HMW) [Daniele A. 2008]. Mannose binding lectin (MBL) is an acute phase serum glycoprotein that is secreted by the liver and present as mixture of dimers and oligomers. MBL deficiency could lead to a chronically activated inflammatory cascade, resulting in insulin resistance [Fernandez-Real 2006]. The aim of this study was to investigate whether Acpr30 and MBL alterations are involved in the pathogenesis of DM1. Material and methods: We selected 22 DM1 patients and 63 age, sex and weight-matched controls. Total serum adiponectin and HMW, MMW and LMW oligomers were evaluated by ELISA, western blot and FPLC; total MBL was evaluated by ELISA. Results: Compared to controls, DM1 patients showed lower concentrations of total adiponectin and its HMW oligomers ($p < 0.05$) as well as that of MBL ($p < 0.05$). Conclusions: The decreased expression of total adiponectin, of its HMW oligomers and of total MBL could explain in part the insulin resistance of DM1 patients. Then adiponectin and MBL could represent new biomarkers in the early

*The Addendum is not part of the original version of this Abstract publication

diagnosis and follow up of insulin resistance in DM1 disease.

A case of maternal phenylketonuria: molecular analysis of neutral amino acid transporter 1 gene

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Introduction: Phenylketonuria (PKU) is a metabolic disease caused by mutations in Phenylalanine hydroxylase (PAH) gene encoding a hepatic enzyme that converts Phenylalanine (Phe) in Tyrosine. The enzyme deficiency leads to an increase of Phe that severely damages the brain if not promptly treated [Daniele A 2009]. Treatment of PKU consists essentially in non compliant diet then was explored a gene therapy approach [Cerreto M 2012]. Elevated plasma levels of Phe during pregnancy exert severe teratogenic effects affecting the morphogenesis and brain development known as maternal PKU syndrome (MSPKU). In this study, we report a case of MSPKU; a baby born from a severe PKU patient with Phe levels of above 1200 umol/l. The patient came to the Campania Center of PKU screening and, despite the genetic counseling, followed no diet therapy and the baby was born affected by MSPKU classical (severe microcephaly, heart defects). Methods: We performed molecular analysis of LAT-1 and PAH genes in both patient and newborn; we designed primes for the amplification of LAT-1 gene and performed from blood sample DNA extraction, PCR reaction and direct sequencing of PAH and LAT-1 genes. Results We highlighted in the mother the presence of two mutations PKU causative: c.1066-11G > A, p.L48S and in the newborn the mutation p.L48S in heterozygosity. The molecular analysis of the LAT-1 gene revealed the presence of the 345C > A polymorphism in both mother and newborn. Conclusions: Nucleotide alterations in the LAT1 gene may play an important role in determining different susceptibility to brain damage in MSPKU patients.

Molecular analysis of PAH gene in italian patients affected by phenylketonuria by DNA high throughput sequencing

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Introduction: Phenylketonuria (OMIM 261600) is the most common disorder of amino acid metabolism caused by phenylalanine hydroxylase (PAH: EC 1.14.16.1) deficiency. Accumulation of Phe causes severe brain damage with mental retardation but an early Phe-restricted diet prevents neurocognitive and developmental damage. In PKU, defective PAH activity results from mutations in the PAH gene. To date, more than 600 mutations have been identified (http://www.pahdb.mcgill.ca). Mutation analysis is important to obtain information both about the expected phenotype and for genetic counseling. In our lab, we perform the molecular diagnosis of HPA with a detection rate of ~ 90% [Daniele 2008, Daniele 2011]. Our aim is to set-up a new and more sensitive strategy using high-throughput sequencing approach. Materials and Methods: From controls and PKU patients identified from neonatal screening, we collected a blood sample (3 ml) in EDTA and extracted DNA using a standard protocol. Using the Primer3 software (http://frodo.wi.mit.edu/primer3/), we designed specific primers encompassing about 100 kb including 20 kb of the promoter region, all exons and introns, and 5 kb of the 3' UTR region of PAH gene. Results Overlapping long-PCR amplicons, ranging in size between 8-12 kb, were generated using a "touch-down" PCR protocol. Each amplicon was individually amplified and purified. After appropriate quality assessment (2100 BioAnalyzer, Agilent) and quantification (Picogreen assay, Invitrogen), the amplification products from the same DNA sample are sequenced. Conclusions: The PCR amplification/high throughput sequencing approach detects both single-nucleotide polymorphisms and CNVs in target genes thereby increasing the spectrum of detected variations within the analyzed gene.