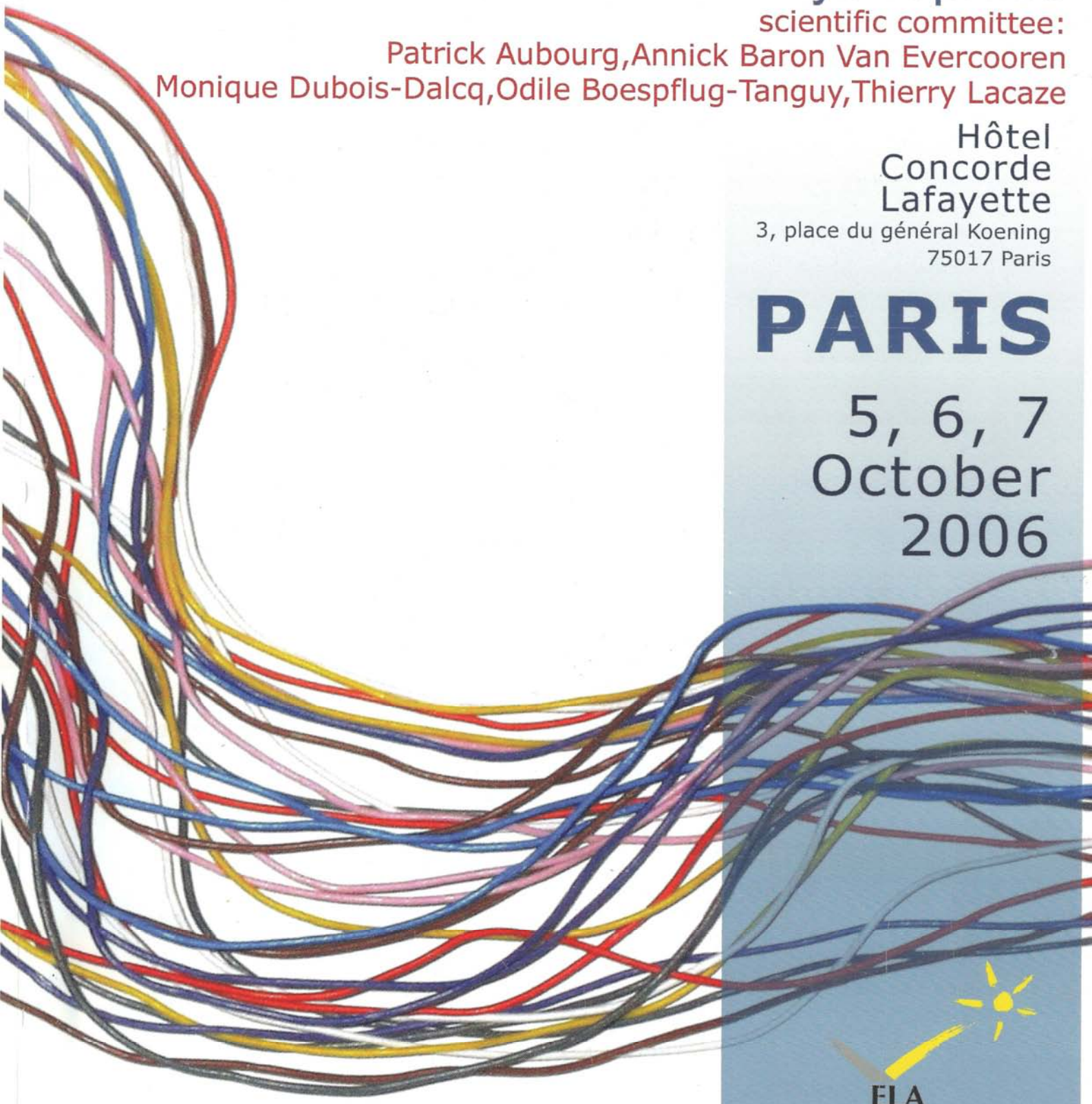
The Vital Function of Myelin Development and Maintenance:

targeting periventricular leukomalacies and leukodystrophies

scientific committee:
Patrick Aubourg,Annick Baron Van Evercooren
Monique Dubois-Dalcq,Odile Boespflug-Tanguy,Thierry Lacaze



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5, 6, 7
October
2006

ELA
Research Foundation

The Myelin Project

Organisation congress committee:
Christian Gex,Rose-Marie Delattre,Julia Alba

ELA FOUNDATION – MYELIN PROJECT (Paris Congress – October 2006)

A recent survey among EUROPEAN LEUKODYSTROPHY ASSOCIATION (ELA) patients and families showed that 80% of them consider medical research as the main objective, a priority that needed all of our attention and support.

To respond to this massive expectation the ASSOCIATION created in early 2005 a foundation, the ELA FOUNDATION, fully dedicated to medical research.

Thanks to the financial support of the FRENCH MINISTRY OF RESEARCH and to the continuous financial commitment of the ELA ASSOCIATION, the ELA FOUNDATION has been able to address right away the broad spectrum of myelin affections beyond the mere although numerous leucodystrophy forms.

The core challenge of the ELA FOUNDATION is quite clear and demanding at the same time: repair myelin and restore impaired functions, thereby sharing the major concern of its MYELIN PROJECT friends and as a matter of fact of all concerned patients and families.

In order to reach this ambitious goal and make progress in the most balanced and effective fashion, the ELA FOUNDATION has formed a Supervisory Board made of patient related members and well known people strongly involved in our cause, all of them distant from the medical field.

This Board operates side to side with a Scientific Committee made of international experts. Its specific mission is twofold: provide guidance for research orientations and select projects to be financed.

This model, with precisely defined respective responsibilities between its two main acting bodies, has rapidly demonstrated its merits and we are now all quite enthused and looking forward to developing the ELA FOUNDATION into a major international and global institution to successfully help fight and eradicate myelin diseases.

The encouraging news, and we need courage to match the never fading patients' and families' courage, is that all of you biologists, doctors or research scientists, share this interest in the ELA FOUNDATION. The expanding number of proposals that have already been submitted within about a year of its creation gives evidence of this phenomenon.

This first international congress of the ELA FOUNDATION – MYELIN PROJECT has been prepared by its highly qualified and fully dedicated Scientific Committee and it is attended by world class participants. It is therefore going to be a high level exchange platform and, we hope, a beginning for new ambitions as well.

Foster additional interest in these diseases, amplify research cooperation by building network communication and expanding whenever possible experience sharing, create a common base of knowledge, these are some of the main benefits we expect from such an international and global endeavour.

This is by all means the goal of the ELA FOUNDATION which will do its best to provide you with the right working environment, together with spirit and motivation, while preserving at the same time focus on patients' interest.

We therefore already invite you to our next venue in Paris the 24th and 25th of March 2007 for a meeting exclusively devoted to patients and their families, to inform them of research advancements.
We are convinced that you will be eager to present to them a simplified summary of your work, accessible for all, and that you will receive full support and gratitude from all ELA members.

On their behalf we sincerely thank you to have accepted our invitation and to be here today. We hope for the Congress to be most informative and productive and wish you all well.

Yours Truly,

Olivier Coutrix
Chairman of the ELA Foundation

Guy Alba
Chairman of the ELA Association

Dear Colleagues

On behalf of the Local Organizing Committee, it is our great pleasure to welcome you to the 1st International Congress of the ELA Foundation taking place in Paris from the 5th to the 7th October, 2006.

Research over the last decade has revolutionized the understanding of diseases affecting the myelin of the central nervous system. Significant advances were made in the elucidation of myelin development and maintenance as well as in the mechanisms of diseases where rapid progress in genetics led to the discovery of gene defects and resulted in new diagnostic tools. Meanwhile neuroregeneration and myelin repair are studied in the context of stem cell biology in animal models where the effect of bioactive molecules, cell and gene therapy can be evaluated in preclinical studies.

The congress program is specifically dedicated to myelin disease of the premature and inherited leukodystrophies which affect mostly children and lead to severe disability and poor quality of life. This congress will provide a wonderful opportunity to meet old and new friends, and, most of all, will facilitate exchange of ideas and knowledge that might lead to new strategies to prevent and/or cure this important group of myelin disorders.

We welcome you to Paris and hope that you will appreciate the scientific sessions and your stay during the Congress.

Respectfully yours

Patrick Aubourg - Odile Boespflug-Tanguy - Monique Dubois-Dalcq - Thierry Lacaze - Anne Baron-Van Evercooren

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THURSDAY, OCTOBER 5, 2006

AFTERNOON PROGRAM

Session 1 : Myelin Development and Repair
Co-Chairs R. Franklin *(Univers. Cambridge - UK)* & A. Baron-Van Evercooren *(Inserm U.546, Paris – France)*

2:00 pm : **Introduction**
Robin FRANKLIN *(Univers. Cambridge - UK)*

2:10 pm : **A role for VGEF-C and VEGFR-3 in neural cell development**
Jean-Léon THOMAS *(Inserm U.711, Paris - France)*

2:40 pm : **Intrinsic regulators of oligodendrocyte progenitor proliferation and differentiation**
Vittorio GALLO *(Center for Neuroscience Research,Washington - USA)*

3:10 pm : **Adult neural stem cells for myelin repair**
Pascale DURBEC *(CNRS-IBDML, Luminy Marseille - France)*

3:40 pm : **Coffee Break**

4 :00 pm : **POSTER SESSION (SESSION 1 ET 2) :**

5:30 pm : **Embryonic stem cells for CNS myelin diseases**
Hans KEIRSTEAD *(Univ.California, Irvine - USA)*

6:00 pm : **Redundant and non-redundant mediators of remyelination - finding the therapeutic targets**
Robin FRANKLIN *(Univ. Cambridge- Cambridge - UK)*

6:30 pm : **Exploring oligodendrogenesis from Human neural stem cells for myelin repair and myelin repair**
A.BARON-VAN EVERCOOREN *(Inserm U.546, Paris – France)*

Concluding remarks
A.BARON-VAN EVERCOOREN *(Inserm U.546, Paris – France)*

8 :30 : **Dinner**

THE VITAL FUNCTION OF MYELIN DEVELOPMENT AND MAINTENANCE : TARGETING PERIVENTRICULAR LEUKOMALACIES AND LEUKODYSTROPHIES

FRIDAY OCTOBER 6, 2006

MORNING PROGRAM

Session 2 : Mechanism of glial cell and axonal pathology in CNS myelin disorders
Co-Chairs : Francesca Aloisi *(Roma - Italy)* & Monique Dubois-Dalcq *(NIH– USA)*

9:00 am : **Myelin/axon interactions: lessons from animal models**
Klaus-Armin NAVE *(Max Planck Institute, Goettingen - Germany)*

9:30 am : **The development of oligodendrocytes in early-onset leukodystrophies**
Ian DUNCAN *(University Wisconsin, Madison - USA)*

10:00 am: **Glycosaminoglycans as signals for remyelination failure**
Larry SHERMAN *(Oregon Health & Science Univ., Portland, Oregon - USA)*

10:30 am **Coffee Break**

11:00 am : **Mechanisms of excitotoxic damage to white matter**
Carlos MATUTE *(Universitad del Pais Vasco, Viszcaya - Spain)*

11:30 am : **Neurotransmitter signalling to oligodendrocytes and its role in white matter disease**
David ATTWELL *(University College London, London - UK)*

12:00 am : **Summary/Concluding remarks**
Monique DUBOIS-DALCQ *(NIH, Bethesda – USA)*

12:15 pm : **Lunch/Buffet**

13:30 pm : **POSTER SESSION (session 3 and 4)**

THE VITAL FUNCTION OF MYELIN DEVELOPMENT AND MAINTENANCE : TARGETING PERIVENTRICULAR LEUKOMALACIES AND LEUKODYSTROPHIES

FRIDAY OCTOBER 6, 2006

AFTERNOON PROGRAM

Session 3 : White matter disease of the premature
Co-Chairs : Thierry Lacaze *(University Alberta, Edmonton -Canada)* & Patrick Aubourg *(INSERM U.745,Paris-France)*

3:30 pm : **White matter disease of the premature**
Thierry LACAZE *(University Alberta, Edmonton -Canada)*

4:10 pm : **MRI of the white matter disease of prematurity**
Mary RUTHERFORD *(Imperial College Medicine, London-UK)*

4:40 pm : **Coffee Break**

5 :00 pm : **White matter of the premature : animal models**
Pierre GRESSENS *(Inserm U.676, Paris-France)*

5:30 pm : **The adaptive immune response in neonatal cerebral white matter damage**
Olaf DAMMANN *(Hannover Medical Scool, Hannover-Germany)*

6:00 pm : **Mechanisms of oligodendrocyte death in the white matter disease of the premature**
Frances JENSEN *(Children's Hospital, Boston-USA)*

6:30 pm : **Concluding remarks**
Patrick AUBOURG *(INSERM U.745,Paris-France)*

8:00 pm **Dinner**

THE VITAL FUNCTION OF MYELIN DEVELOPMENT AND MAINTENANCE : TARGETING PERIVENTRICULAR LEUKOMALACIES AND LEUKODYSTROPHIES

SATURDAY OCTOBER 7, 2006

MORNING PROGRAM

Session 4 : Genetics and Therapeutics of Leukodystrophies
♦Astrocytes, a new target for leukodystrophies
Chair : Jutta GARTNER *(University Göttingen, Göttingen – Germany)*

8:30 am : **Introduction**
Jutta GARTNER*(University Göttingen, Göttingen – Germany)*

8:35 am : **Alexander disease : a large clinical spectrum**
Diana RODRIGUEZ ***(****Hôpital Trousseau, Paris, France)*

8:50 am : **Alexander disease : a protein aggregation pathology**
Danielle PHAM DINH *(INSERM U 546, Paris- France)*

9:15 am : **EIF2B Deficiency : Selective Vulnerability of CNS Glial Cells**
Raphael SCHIFMANN *(NIH, Bethesda – USA)*

9:40 am : **EF2B deficiency : a stress-induced pathology ?**
Orna ELROY STEIN *(Tel Aviv University, Tel Aviv -Israel)*

10:00 am : **The cystic leukoencephalopathies**
Enrico BERTINI *(Bambino Gesu' Children's Research Hospital, Rome – Italy)*

10:15 am : **MLC1 leukoencephalopathy**
Elena AMBROSINI (*Instituto Superiore di Sanita, Roma – Italy)*

10:30 am : **Coffee Break**

♦Therapeutic Approaches in Leukodystrophies
Chair : Odile Boespflug Tanguy *(Inserm U.384, Clermont Ferrand –France)*

11:00 am : **Introduction**
Odile BOESPFLUG-TANGUY *(Inserm U.384, Clermont Ferrand –France)*

11:05 am : **Screening Compounds to Ameliorate EIF2B Deficiency**
Graham PAVITT *(University of Mancheste, Manchester - UK)*

11:35 am **: Gene and cell therapy in leukodystrophies**
Patrick AUBOURG *(Inserm U.745,Paris - France)*

11:50 am : **Combined therapies for the treatment of Krabbe disease**
David WENGER *(Jefferson Medical College, Philadelphia - USA)*

12:05 am : **Inhibiting PLP gene mutations**
Franca CAMBI *(University Kentucky, Lexington - USA)*

12:20 am : **Concluding remarks**
Jutta GARTNER *(University Göttingen, Göttingen – Germany)*

12:30 pm : **Lunch/Buffet**

SESSION 1 : MYELIN DEVELOPMENT AND REPAIR

Co-Chairs
R. FRANKLIN (Univers.Cambridge-UK)
A. BARON-VAN EVERCOOREN (Inserm U.546, Paris-France)

INTRODUCTION

Robin FRANKLIN

Cambridge Center for Brain Repair & Department of Veterinary Medicine
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A ROLE FOR VEGF-C AND VEGFR-3 IN NEURAL CELL DEVELOPMENT

Jean-Léon Thomas1,2
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2Université Pierre & Marie Curie, Faculté de Médecine Pitié Salpêtrière, IFR 70, Paris, F-75005France

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We are investigating the molecular control of oligodendrocyte development, in particular the possible role of vascular growth factors. The development of the central nervous system (CNS) relies on continuous interactions between neural and endothelial cells, including exchange of common chemical signals such as VEGF-A (vascular endothelial growth factor-A), a member of VEGF family of vascular growth factors which has been implicated in neural development and neurodegenerative diseases like ALS. We are studying the lymphangiogenic factor VEGF-C (Vascular Endothelial Growth Factor-C). We have shown expression of both VEGF-C and its specific receptor VEGFR-3 by complementary populations of neural cells in the embryonic brain. Genetic ablation of Vegfc in frogs and mice results in decreased proliferation of neural progenitors, in the absence of vascular defects in the CNS. The effect of VEGF-C on neural cells is direct and probably mediated by VEGFR-3. In the oligodendroglial lineage, VEGF-C stimulates proliferation and migration of subpopulations of oligodendrocyte precursor cells (OPCs) expressing VEGFR-3. Altogether these findings demonstrate a requirement of VEGF-C by subpopulations of neural precursors during embryonic development (Le Bras et al., 2006).

INTRINSIC REGULATORS OF OLIGODENDROCYTE PROGENITOR PROLIFERATION AND DIFFERENTIATION

Vittorio Gallo

Center for Neuroscience Research, Children's National Medical Center, Washington, DC, USA

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In the postnatal brain, oligodendrocytes derive from immature oligodendrocyte progenitor cells (OPCs) that divide in the subventricular zone (SVZ), and migrate to white matter regions to differentiate and myelinate axons. SVZ progenitors that give rise to oligodendrocytes express the proteoglycan NG2. Oligodendrocyte maturation is a continuum of cellular events that require synchronized and timely expression of numerous genes that coordinate each phase of OPC development. Therefore, in order to promote oligodendrocyte regeneration and remyelination after demyelination, it is of primary importance to identify the key molecular regulators of OPC proliferation and differentiation. A crucial determinant of progenitor proliferation is the duration of the cell cycle, which is tightly regulated by kinase signaling mechanisms. We have previously demonstrated that cdk2 plays an important role in the control of glial progenitor proliferation by regulating G1/S transition. We analyzed a Cdk2-/- mouse mutant and show that, in adult (postnatal day 60; P60) neurogenic areas, including the SVZ, loss of Cdk2 reduces NG2-expressing progenitor cell proliferation and number. Conversely, no changes in $NG2^+$ cell proliferation and number were observed at P8. Loss of Cdk2 also promoted cell differentiation in P60 brain, by reducing the number of $NG2^+nestin^+$ cells, and by increasing the number of $Nkx2.2^+$ committed oligodendrocytes and Dcx^+ neuroblasts in the SVZ and rostral migratory stream. Consistent with these findings, analysis of P60 SVZ Cdk2-/- cell cultures demonstrated inhibition of neurosphere formation and enhanced neural cell differentiation. No effects were observed in P8 cultures. Immunoblotting revealed that, in P8 $Cdk2^{-/-}$ SVZ tissue, expression of several cell cycle regulators, including Cdk4, Rb and E2F-1 was significantly increased. Conversely, in P60 SVZ, the levels of most cell cycle regulatory proteins were similar in $Cdk2^{-/-}$ and WT mice, with the exception of increased p21 expression in Cdk2-/-. siRNA Cdk4 knockdown in P8 $Cdk2^{-/-}$ neurospheres resulted in a decrease in cell proliferation and in neurosphere formation similar to that observed in P60 cells.

We used microarray analysis to identify trancription factors that regulate OPC differentiation. Analysis of oligodendrocyte lineage cells FACS-purified from CNP-EGFP transgenic mice revealed Sox17 gene expression to be coordinately regulated with that of four myelin genes during postnatal development. In $CNP\text{-}EGFP^+$ cells, Sox17 mRNA and protein levels transiently increased between postnatal day 2 (P2) and P15, with white matter $O4^+$ pre-oligodendrocytes expressing greater Sox17 levels than $Nkx2.2^+$, $NG2^+$ or $GalC^+$ cells. In spinal cord, Sox17 protein expression was undetectable in the pMN domain between E12.5 and E15.5, but was evident in $Nkx2.2^+$ and $CC1^+$ cells. In cultured oligodendrocyte progenitor cells (OPCs), Sox17 levels were maximal in $O4^+$ cells, and peaked during the phenotypic conversion from bipolar to multipolar. Parallel increases in Sox17 and p27 occurred prior to MBP protein expression, and Sox17 upregulation was prevented by conditions inhibiting differentiation. Sox17 downregulation with siRNAs increased OPC proliferation and decreased lineage progression following mitogen withdrawal, whereas Sox17 overexpression

in the presence of mitogen had opposite effects. Sox17 overexpression enhanced myelin gene expression in OPCs and directly stimulated MBP gene promoter activity.

In conclusion, our findings demonstrate a crucial role of Cdk2 in adult SVZ progenitor proliferation and differentiation, and indicate that the function of intrinsic regulators of progenitor cell proliferation changes during postnatal development of the SVZ. Our results also support important roles for Sox17 in controlling both OP cell cycle exit and differentiation.

ADULT NEURAL STEM CELLS FOR MYELIN REPAIR

Pascale Durbec, Myriam Cayre,Karine Magalon, Cristina Cantarella.

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Stem cells have been shown to persist in the adult mammalian brain where they are predominantly committed to a neuronal fate. Recent studies presented evidence that endogenous stem cells from the adult subventricular zone (SVZ) could constitute a source of cells for repair in neurodegenerative diseases, including demyelinating pathologies. In multiple sclerosis, the most common demyelinating disease of the central nervous system (CNS), death of oligodendrocytes leads to demyelination throughout the entire white matter, with areas of predilection such as the periventricular fibre tracts. In this context, one possible therapeutic strategy is to directly mobilize endogenous stem cells to the lesions. Although demyelinated brain lesions produce cues attracting progenitor cells from the SVZ, these spontaneous repair attempts are too limited to allow full recovery. In this regard, it seems important to find out what factors are limiting in this repair process. We first evaluated the migration potential of SVZ cells in adult brain outside their physiological migration pathway and their potentiality to generate oligodendrocytes. We transplanted neural progenitor cells from the SVZ into the cingulum of unlesioned wild type as well as myelin-deficient recipient adult mice, and analyzed the migration of these cells in the brain. We showed that grafted SVZ progenitor cells perform long distance migration along white matter tracts, and are able to differentiate into mature oligodendrocytes in the cingulum and the corpus callosum. Our results clearly indicate that SVZ neural stem/progenitor cells can achieve widespread dispersion and differentiation into oligodendrocytes in the adult brain, supporting the potential use of these cells for myelin repair. Thus, since migration and oligodendrocyte differentiation are not limiting in the repair process, we are currently testing factors and conditions capable of enhancing SVZ cell recruitment to the lesions. In this context, environment enrichment appears as a broad non-invasive strategy of potential interest. Using rodent models of multiple sclerosis such as experimental autoimmune encephalomyelitis (EAE) and lysolecithine injection, we showed that enriched living conditions promoted SVZ cell proliferation and increased the number of SVZ cells recruited into demyelinated structures. Among these cells higher proportion adopted oligodendrocytic fate. Furthermore, environment enrichment also reduced functional impairment in EAE mice.

EMBRYONIC STEM CELLS FOR CNS MYELIN DISEASES

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Demyelination contributes to loss of function following spinal cord injury, and so a potential therapeutic strategy involves replacing myelin forming cells. Here, we show that human embryonic stem cells (hESCs) can be directed in their differentiation into high purity oligodendrocyte progenitors (OPCs), and that transplantation of hESC -derived OPCs into adult rat spinal cord injuries enhances remyelination and promotes recovery of motor function. We hypothesize that hESC – derived OPCs promote neural repair and behavioral recovery through multiple mechanisms, including transplant-mediated remyelination and transplant-mediated effects on endogenous cells. We present both efficacy and safety data concerning the use of this cell population in transplant regimes, and regulatory concerns regarding the translation of stem cell technologies into human treatments.

REDUNDANT AND NON-REDUNDANT MEDIATORS OF REMYELINATION – FINDING THE THERAPEUTIC TARGETS

Robin J.M. Franklin

Cambridge Centre for Brain Repair, University of Cambridge, Cambridge CB3 OES, UK

Remyelination, the process by which new myelin sheaths are restored to demyelinated axons, represents one of the most compelling examples of adult multipotent progenitor cells contributing to regeneration of the injured CNS. This process can occur with remarkable efficiency in both clinical disease, such as multiple sclerosis, and in experimental models, revealing an impressive ability of the adult CNS to repair itself. However, the inconsistency of remyelination in multiple sclerosis and other myelin diseases, and the loss of axonal integrity that results from its failure, makes enhancement of remyelination an important therapeutic objective. Identifying potential targets will depend on a detailed understanding of the cellular and molecular mechanisms of remyelination. This talk will introduce the concept the environmental signalling factors that govern remyelination exhibit a large degree of redundancy and that rather depending on single critical factors, a matrix of signalling events is required for the successful completion of remyelination.

EXPLORING OLIGODENDROGENESIS FROM HUMAN NEURAL STEM CELLS FOR MYELIN REPAIR.

A. Baron-Van Evercooren*, B. Nait Oumesmar, D. Buchet, C. Garcia, C. Maire, C. Deboux.

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In myelin diseases such as multiple sclerosis (MS) and leucodystrophies, the destruction of oligodendrocytes and their myelin alters neural conduction leading to subsequent axonal loss, and causing severe neurological deficits. Investigations in animal models indicate that cell transplantation or activation of endogenous remyelination can result in anatomical and functional recovery of acute myelin lesions. In this respect, the discovery of multipotent stem cells and early progenitors in the embryonic and adult rodent brain has pointed to novel therapies to promote myelin repair. However, little is known on the potential of repair of neural stem cells (NSC) in humans. Experimental demyelination in rodents has highlighted the activation of the subventricular zone (SVZ) in response to demyelination, and the involvement of the newly generated progenitors in the repair process. Our recent data indicate that such as in rodents, activation of gliogenesis occurs in the human SVZ in MS, and suggest the mobilization of these early progenitors to peri-ventricular lesions where they could be the source of new oligodendrocyte progenitors. These cells could be potential targets for therapeutic strategies to enhance myelin repair in the diseased CNS. Moreover they may serve as vehicles for protein delivery allowing targeting of gene therapy to myelin lesions. Cell therapy may be an alternative strategy to enhance myelin repair. Even though primate NSC can generate oligodendrocytes, little is known about the factors that govern their differentiation into the oligodendroglial fate and consequently, the proportion of primate oligodendroglial cells available for cell therapy remain insufficient for transplantation. We are exploring several avenues (regional specificity, immuno-selection and transcription factor over-expression,) to derive cell populations enriched in oligodendrocyte progenitors from primate fetal brain (8-10 week post gestation), and will discuss the potency of each of these strategies to promote the generation of human oligodendrocytes for cell replacement therapies for myelin

CONCLUDING

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SESSION 2 : MECHANISM OF GLIAL CELL AND AXONAL PATHOLOGY IN CNS MYELIN DISORDERS

Co-Chairs
F. ALOISI (Roma-Italy) & M.DUBOIS-DALCQ (NIH, Bethesda-USA)

MYELIN/AXON INTERACTIONS : LESSONS FROM ANIMAL MODELS

Klaus Armin NAVE (Max Planck Institute, Göettingen-Germany)

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THE DEVELOPMENT OF OLIGODENDROCYTES IN EARLY-ONSET LEUKODYSTROPHIES

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The oligodendrocyte progenitor cell (OPC) is one of the most well studied progenitors of the body. The differentiation of these cells into mature, myelin-bearing oligodendrocytes requires the intricate temporal expression of myelin genes and oligodendrocyte-associated genes, for normal development to occur. However, even prior to these molecular events, the OPC's arise from neural stem cells which in turn are derived during early development from the embryonic stem (ES) cell. Thus is appears that there are two major phases of development of oligodendrocytes, from ES cell to OPC and from OPC to the mature oligodendrocyte. In each phase, multiple intermediate stages are found that are identified by a battery of markers. Definition of the stages from the ES cell to OPC is as yet less complete. In general, most of the characterized leukodystrophies have been found to result from a derangement of development from OPC to oligodendrocyte or from abnormalities of the mature cell. I will begin by discussing how mutations of the PLP gene affect oligodendrocyte differentiation and survival in the X-linked mutants. I will contrast that with what is known in the autosomal recessive mutants. The latter involve mutations in known myelin genes; other myelin-developmental disorders in higher species may involve other aspects of the normal differentiation and distribution of OPC's and these will be discussed. Finally, in certain leukodystrophies, OPCs mature into myelinating oligodendrocytes that eventually degenerate with resultant demyelination. The knowledge that has been gained by studying early development of OPC's and the role of transcription factors such as the oligodendrocyte genes, will likely lead to the discovery of the origin of uncharacterized childhood leukodystrophies.

GLYCOSAMINOGLYCANS AS SIGNALS FOR REMYELINATION FAILURE

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Demyelination is the hallmark of numerous neurodegenerative conditions. Oligodendrocyte progenitors, which normally mature into myelin-forming oligodendrocytes, are typically present around demyelinated lesions but sometimes fail to remyelinate affected axons, especially in chronic lesions. We found that the glycosaminoglycan hyaluronan (HA) accumulates in demyelinated lesions from patients with multiple sclerosis, in mice with experimental autoimmune encephalomyelitis, in spinal cord injuries, in infants with periventricular white matter injury, and during the course of normal aging. HA accumulation in these lesions is linked to elevated levels of hyaluronan synthase 2, especially in reactive astrocytes, as well as elevated levels of the CD44 transmembrane HA receptor. A high molecular weight (MW) form of HA synthesized by astrocytes accumulates in chronic demyelinated lesions. This form of HA inhibits the proliferation of astrocytes and blocks remyelination following lysolecithin-induced white matter demyelination. Oligodendrocyte progenitors accrue and fail to mature into myelin-forming cells in demyelinating lesions where high MW HA is present. Furthermore, the addition of high MW HA to oligodendrocyte progenitor cultures reversibly inhibits both progenitor cell maturation and neural stem cell differentiation at lower levels, while it inhibits progenitor cell proliferation at higher levels. Degrading HA in astrocyte-oligodendrocyte progenitor co-cultures promotes oligodendrocyte maturation. High MW HA may therefore contribute significantly to remyelination failure by preventing both the expansion and maturation of oligodendrocyte progenitors or neural stem cells that are recruited to demyelinating lesions.

MECHANISMS OF EXCITOTOXIC DAMAGE TO WHITE MATTER

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Glutamate kills neurons by excitotoxicity which is caused by sustained activation of glutamate receptors. In recent years, it has been shown that glutamate can also be toxic to white matter oligodendrocytes and to myelin by this mechanism. In particular, glutamate receptor-mediated injury to these cells can be triggered by activation of AMPA, kainate and NMDA glutamate receptor types. Thus, these receptor classes, and the intermediaries of the signal cascades they activate, are potential targets for drug development to treat white matter damage in acute and chronic diseases. In addition, alterations of glutamate homeostasis in white matter can determine glutamate injury to oligodendrocytes and myelin. Astrocytes are responsible for most glutamate uptake in synaptic and non-synaptic areas and consequently, are the major regulators of glutamate homeostasis. Activated microglia in turn may secrete cytokines and generate radical oxygen species, which impair glutamate uptake and reduce the expression of glutamate transporters. Finally, oligodendrocytes, also contribute to glutamate homeostasis. My presentation aims at summarizing the current knowledge about the mechanisms leading to oligodendrocyte cell death and demyelination as a consequence of alterations in glutamate signalling, and their clinical relevance to disease. A thorough understanding of how oligodendrocytes and myelin are damaged by excitotoxicity will generate knowledge that can lead to improved therapeutic strategies to protect white matter. Reference Matute C, Domercq M, Sanchez-Gomez MV (2006) Glutamate-mediated glial injury: mechanisms and clinical importance. Glia 53:212-224.

NEUROTRANSMITTER SIGNALLING TO OLIGODENDROCYTES AND ITS ROLE IN WHITE MATTER DISEASE

Ragnhildur Káradóttir, Nicola Hamilton, Yamina Bakiri, Pauline Cavelier, Linda H. Bergersen & **David Attwell***

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Glutamate-mediated damage to oligodendrocytes contributes to mental or physical impairment in periventricular leukomalacia (pre- or perinatal white matter injury leading to cerebral palsy), spinal cord injury, multiple sclerosis and stroke. Unlike neurons, white matter oligodendrocytes have been reported to lack NMDA receptors and it has been suggested that glutamate damages oligodendrocytes, especially their precursor cells, by acting only on calcium-permeable AMPA/kainate receptors. We have shown that precursor, immature and mature oligodendrocytes, in the white matter of the cerebellum and corpus callosum, exhibit NMDA-evoked currents, mediated by receptors which are blocked only weakly by Mg2+, and which may contain NR1, NR2C and NR3 subunits. NMDA receptors are present in the myelinating processes of oligodendrocytes, where the small intracellular space could lead to a large rise of intracellular ion concentration in response to NMDA receptor activation. Simulating ischa emia leads to an inward current developing in oligodendrocytes, which is partly mediated by NMDA receptors. Blocking ischaemic activation of NMDA receptors reduces the loss of the action potential occurring in myelinated fibres. These results point to NMDA receptors of unusual subunit composition as a novel therapeutic target for preventing white matter damage in a range of diseases. The role of neurotransmitter signalling to oligodendrocyte precursor cells will also be discussed.

CONCLUDING

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SESSION 3 : WHITE MATTER DISEASE OF THE PREMATURE

Co-Chairs
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WHITE MATTER INJURY IN PRETERM BABIES

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Premature births represent around 7% of all births, but account for more than 80% of all perinatal complications and death. Survival of very preterm infants has increased because of the widespread use of surfactant treatment for respiratory distress syndrome, together with antenatal glucocorticoids and new ventilator strategies. However, these infants are at high risk for long term sequelae of both the brain (cerebral palsy) and the lungs (bronchopulmonary dysplasia). These substantial morbidities have important ramifications for: (i) the healthcare system in terms of costs and resource use; (ii) the families both emotionally and financially; and, (iii) society in general in terms of support and integration of persons with lifelong disabilities.

Several observational studies strongly suggest that the risk of developing brain injury is dependent on antenatal factors, with the highest risk in infants born to mothers with intrauterine infection or chorioamnionitis. White matter injury (WMI) and cystic periventricular leukomalacia (PVL) - the most severe form of WMI - are recognized as a major identifiable risk factor for cerebral palsy (CP) in very preterm infants. One in four children with WMI (ventricular dilation, persistent echodensities, or cystic periventricular leukomalacia) eventually develop CP. There is accumulating evidence suggesting that intrauterine infection/perinatal inflammation and inflammatory cytokines released during the course of chorioamnionitis play a key role in the development of white matter injury (WMI). Exposure to bacterial lipopolysaccharides during pregnancy induces WMI in several animal models and cystic PVL and CP are more frequent in infants born to mothers with clinical or histological chorioamnionitis. A recent meta-analysis examining the relationship between chorioamnionitis and cerebral palsy demonstrates that clinical chorioamnionitis is significantly associated with CP (RR: 1.9; 95% CI: 1.4-2.5). The same-meta-analysis also shows that cystic PVL is significantly associated with both clinical (RR: 3.0; 95% CI: 2.2-4.0) and histological (RR: 2.1; 95% CI: 1.5-2.9) chorioamnionitis.

It has been suggested that the fetal systemic inflammatory response mediated by pro-inflammatory cytokines may participate in the onset of labor as well as PVL and CP. Elevated amniotic fluid or cord blood IL-6, IL-8, and TNF alpha concentrations have been found significantly associated with cystic PVL and CP. Recent studies performed on a small number of patients have also shown that the presence of funisitis, the histological counterpart of the fetal inflammatory response syndrome, is a strong and independent risk factor for the subsequent development of CP. This finding suggests that it is the fetal, rather than the maternal, inflammatory reaction that predisposes to CP. In addition, the white matter of the very premature baby remains a target for an inflammation-triggered insult for the first weeks after birth. Indeed, PVL can develop after late-onset neonatal sepsis or necrotizing enterocolitis and late-onset neonatal sepsis has been recently found to be an independent predictive marker of CP. Finally, PVL incidence seems to be reduced in infants whose mother received glucocorticoids before delivery. All these findings strongly suggest that the pathophysiological process accounting for WMI and PVL begins during intrauterine life and is related to the exposure to intraamniotic inflammation and the triggering of a fetal inflammatory response. The lesion occurs when white matter is immature and there is a dominance of oligodendrocytes precursors. These different degrees of WMI (from diffuse

myelinisation disturbance to classic cystic PVL) are the result of damage to oligodendrocyte progenitors and the disruption of their full maturation to myelinating cells.

While the relationships between perinatal inflammation, the early occurrence of WMI, and the subsequent development of CP are well established, there is little information available about a possible association between perinatal infection/inflammation and the subsequent occurrence of cognitive limitations without motor deficit in children born prematurely. Whether such a relationship exists has become an important concern because the incidence of cystic PVL has dramatically decreased in very premature infants. The rate of CP among children born with a birth weight below 1250 grams is also lower today than it was years ago (In Alberta: 13% in 1975 versus 5% in 1997). However, whereas the vast majority of these infants are nowadays discharged without ultrasonographic evidence of brain damage, the rate of cognitive impairment, defined as a Mental Developmental Index (MDI) < 70 or -2SD below the mean, appears to be rising (for instance in Alberta: 13% in 1975-78 versus 30% in the mid 1990's of children born less than 1250 grams have a MDI < 55; 26% in mid 1980's versus 48% in mid 1990's of children born less than 750 grams have a MDI < 70). This alarming trend suggests that either structural abnormalities are present but undetectable by routine head ultrasounds or cognitive limitation correlates more with abnormal function than head ultrasounds-diagnosed damage. In this regards, Magnetic Resonance Imaging (MRI) could be more sensitive than head ultrasounds in predicting cognitive impairment in children born prematurely.

Further development in animal models will provide insight in the mechanisms leading to WMI whereas improvement in MRI techniques in animal and human will assist in understanding the different patterns of WMI as well as predicting long-term outcome. Information gathered from animal models, MR imaging, and long-term follow-up will underpin the development of various neuroprotective strategies that will be soon evaluated through clinical trials to prevent perinatal brain injury in very preterm infants.

MRI OF THE WHITE MATTER DISEASE OF PREMATURITY.

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Traditionally white matter disease following preterm birth has included two disorders; periventricular leucomalacia and haemorrhagic venous infarction. Both diseases are identifiable with cranial ultrasound and usually result in a poor neurodevelopmental outcome for the child, initially characterised by motor impairment.

Recent long term follow up studies have shown that as many as 40% of survivors from very preterm delivery have significant cognitive impairment in the absence of motor abnormalities. The neuroanatomical correlates for these neurocognitive impairments are not well understood. One current hypothesis is that they are associated with abnormal cortical development, which occurs secondary to diffuse white matter injury.

It is now possible to use magnetic resonance (MR) imaging to assess the preterm brain. Recent MR studies have shown different abnormalities within the white matter that were not detected with routine ultrasound studies. The two appearances have been described diffuse abnormal signal intensity, DEHSI and punctate white matter lesions.
Current research is aimed at establishing the significance of these "new" appearances on the developing brain and their significance for short term and long -term outcome in the child.

More difficult to undertake are studies designed to investigate the aetiology of these new diseases and in particular to ask whether they represent milder forms of periventricular leucomalacia.

WHITE MATTER OF THE PREMATURE: ANIMAL MODELS

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The aetiology of white matter injury in human neonates has been widely described as multifactorial rather than linked solely to cardiovascular instability and hypoxia-ischemia. The many pre-conceptional, prenatal, perinatal, and postnatal factors potentially implicated in the pathophysiology of neonatal white matter lesions include hypoxia-ischemia, endocrine imbalances, genetic factors, growth factor deficiency, abnormal competition for growth factors, overproduction of free oxygen radicals, maternal infection with overproduction of cytokines and other pro-inflammatory agents, exposure to toxins, maternal stress, and malnutrition. Although some of these potentially noxious factors may suffice to permanently injure the developing brain, some researchers have developed a two-hit hypothesis in which early exposures increase the susceptibility of the brain to subsequent insults.

The development and characterization of distinct yet complementary animal models should help to unravel the complex cellular and molecular pathophysiological mechanisms underlying perinatal white matter lesions. In available animal models or in *in vitro* paradigms, the insults most often used to induce white matter damage or oligodendrocyte cell death, respectively, are generally **hypoxia, hypoxia-ischemia, infection, inflammatory factors, oxidative stress, excitotoxic agents, or combined insults**. The relevance of white matter damage produced in these animal models to human white matter injury is largely based on neuropathological data although some recent studies are also based on MRI parameters or on neurological and behavioural deficits.

These animal models have permitted to identify some of the potentially key cellular (pre-oligodendrocytes, microglia-macrophages, mast cells, subplate neurons …) and molecular (glutamate, cytokines and other inflammatory mediators, reactive oxygen species …) players involved in the pathophysiology of perinatal white matter damage. These studies have also provided experimental evidence supporting a multiple-hit hypothesis for perinatal white matter damage. Furthermore, they have delineated some potential target for neuroprotection and identified good candidate drugs (melatonin, topiramate, anti-inflammatory drugs, anti-oxidant agents, growth factors and growth factor modulators …) for pre-clinical testing and, eventually, clinical testing.

Future studies should incorporate in a larger extent imaging and behavioral studies to facilitate comparisons with the human situation. Although it is clear that studies with rodents, due to their cost and availability, are absolutely necessary to generate and test multiple hypotheses, the use of larger models such as sheep, pigs and monkeys will remain a key step to test in a more reliable fashion the relevance of the obtained data for the human situation.

THE ADAPTIVE IMMUNE RESPONSE IN NEONATAL CEREBRAL WHITE MATTER DAMAGE

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MECHANISMS OF OLIGODENDROCYTE DEATH IN WHITE MATTER DISEASE OF THE PREMATURE

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CONCLUDING

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SESSION 4-1 : GENETICS AND THERAPEUTICS OF LEUKODYSTROPHIES

ASTROCYTES,A NEW TARGET FOR LEUKODYSTROPHIES

Chair
Jutta GARTNER (Univers. Göttingen, Göttingen-Germany)

INTRODUCTION

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ALEXANDER DISEASE: A LARGE CLINICUM SPECTRUM

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AGGREGATES OF MUTATED GFAP FORM AGGRESOMES OR DISAGGREGATE IN AN ASTROCYTIC MODEL OF ALEXANDER DISEASE

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Alexander disease (AXD)—a rare neurodegenerative disorder characterized by large cytoplasmic aggregates in astrocytes and myelin abnormalities—is caused by dominant mutations in the gene encoding glial fibrillary acidic protein (GFAP), the main intermediate filament (IF) protein in astrocytes. We tested the effects of several mutations associated with AXD, as well as putative non-pathogenic polymorphisms of the GFAP gene, in cells transiently expressing GFAP fused to green fluorescent protein (GFP). The human SW13 cells (deficient in IF proteins) and mouse astrocytes were used as in vitro models:

- As expected, the four GFAP polymorphisms tested (D292N, P44L, V112I, E220Q, and D154N) behave as wild type GFAP, forming essentially an apparently normal network in astrocytes as well as in SW13 cells.
- 4 published pathogenic mutations located in the rod domain (R239H, R79H, V87I, L235P) behave similarly, forming a network, aggregates and network, or only aggregates in astrocytes; and only aggregates in SW13 cells. However, R416W, located in the tail domain forms only a network and never aggregates, both cell types.
- 2 new mutations located in the tail-domain, R406 and T412, behave differently from rod-domain mutations (and from R416W mutation), mostly forming very small and diffuse aggregates, disrupting the endogenous network. These results indicate a particular effect of mutations in this part of the molecule, implying interaction of the tail domain with another domain of GFAP, or with other unknown protein partners; whereas mutations affecting the rod domains are directly involved in an abnormal polymerisation of GFAP monomers.

In astrocytes from wild-type, GFAP-, and vimentin-deficient mice, mutated GFAP-GFP (R236H and R79H) may aggregate or form a network, depending on qualitative and quantitative interactions with normal IF partners. Interestingly, vimentin revealed chaperone-like molecule properties allowing formation of a normal network with mutated GFAP.

Using a proteasome inhibitor, the ubiquitination of aggregates was highlighted, indicating an effort to eliminate abnormally folded proteins by the ubiquitine-protéasome system, and suggesting that pathophysiological mechanisms involved in other neurodegenerative disorders related to protein aggregation contribute also to AXD.

Time-lapse recordings of living astrocytes showed that aggregates of mutated GFAP-GFP coalesced into aggresomes associated with cell death or disappeared, which was associated with astrocyte survival. Since aggregation of mutated GFAP was dynamic and reversible, therapeutic approaches may be possible, we are currently testing one of them (geldanamycin).

Our research project comprises the following steps:

- Proteomics and peptidomic approaches (MALDI-TOFF, coll. with R. Stocklin, Atheris Laboratories, Geneva, Switzerland; 2D electrophoreses and/or nano LC MS/MS Post-Genomique Platform (P3S), University Pierre et Marie Curie de Paris 6.
- Inhibition of astrocyte death using XIAP (coll. with S. Gandhour, CNRS Strasbourg)
- Targeting mutated GFAP mRNAs by RNA interference (Collaboration with J. Mallet et A. Privat, Inserm, Paris, Montpellier)
- Generation of an AXD in vivo model: Knock-in mice expressing GFAP mutations (GIS-ICS)

Our results could have potentially therapeutic applications for AXD as well as for other leukodystrophies linked to a primary defect in astrocytes such as *MLC1* and *EIF2B* related diseases. They could also be useful in assessing the role of the non-myelinating glial cells in the pathophysiology of other leukodystrophies, as well as in other situations such as energy metabolism impairment, CNS injuries, infections or abnormal protein accumulation.

EIF2B DEFICIENCY: SELECTIVE VULNERABILITY OF CNS GLIAL CELLS

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Mutations in eukaryotic translation initiation factor 2B (eIF2B) cause a common leukodystrophy, childhood ataxia with CNS hypomyelination/vanishing white matter disease or CACH/VWM. This leukodystrophy is associated with a new onset of progressive childhood neurological disorder. It is also characterized by typical abnormalities on head MRI that are present even in pre-symptomatic children. The mechanism that underlies the typical CACH/VWM MRI image is not well understood. Myelin of the CNS is mostly affected, but in some cases hypomyelination and axonal loss of the peripheral nerves is also present. In the brain, oligodendrocytes and astrocytes seem to be primarily involved. The oligodendrocytes appear to be initially increased in number but with time they undergo progressive "foamy" enlargement that likely reflects accumulation of myelin proteins in the ER, followed by cellular death. The astrocytes are often decreased in number initially but they are also individually hypertrophied with atypical changes seen on glial acidic fibrillary protein (GFAP) immunostaining. It is possible that astrocyte dysfunction is related to the abnormal initial MRI image in patients with CACH/VWM. The dysfunction of these glial cells is also likely to cause the decreased CSF asialotransferrin to transferrin ratio seen in eIF2B-mutated patients. Interestingly, CACH/VWM patients with no eIF2B mutations have normal asialotransferrin to transferrin ratio suggesting a different mechanism of diseases in these patients. Secondary axonal damage is associated with white matter rarefaction (cavitation) and progressive neurological deficit. Because of the relatively small reduction in the GEF activity of mutated eIF2B, it is likely that therapy with small molecules is possible in CACH/VWM. Such therapy should enhance eIF2B activity and may be effective if applied in the early stage of the disease before significant axonal damage occurs.

EIF2B DEFICIENCY: A STRESS-INDUCED PATHOLOGY?

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Eukaryotic translation initiation factor 2B (eIF2B) which is critical for translation initiation under normal conditions, has a critical role in regulation of protein synthesis in response to various cellular stresses. eIF2B-related leukodystrophy, also called CACH or VWM, is a fatal autosomal recessive genetic disease caused by minor loss-of-function mutations in either one of the five genes encoding eIF2B subunits, □□□□□or□. Since the clinical symptoms of the disease deteriorate upon physiological stress, we aim at studying the stress response of eIF2B-mutated cells. We found that primary fibroblasts from CACH/VWM have abnormal stress response compared to primary fibroblasts from normal individuals. Since we are specifically interested in the effect of physiological stress on protein synthesis in brain-related cells, a cellular model was generated by modulation of eIF2B in the DDR1 rat oligodendrocyte-derived cell line. The abnormal stress response of these eIF2B modulated cells will be discussed. To further study the effect of eIF2B deficiency on hypomyelination and to learn about the importance of normal stress response to efficient re-myelination, we generated a knock-in (KI) mouse model. The animal model will enable to follow the progression of the disease under various environmental conditions, and will be imperative for screening of potential therapeutic drugs.

THE CYSTIC LEUKOENCEPHALOPATHIES

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A number of leukoencephalopathies have been described, and the primary defects have been identified in several as a result of the advances in genetics and molecular biology. However, the pathophysiological mechanisms in most of the cystic leukoencephalopathies remain unknown at this time. A first disease called „Leukoencephalopathy with Swelling and a Discrepantly Mild Clinical Course,‰ later called „Megalencephalic Leukoencephalopathy with Subcortical Cysts,‰ was described in 1995 by van der Knaap et al. This entity is characterized by dramatic MRI changes but mild clinical manifestations initially, with development of macrocephaly and functional deterioration later in the course. Mutations in the MLC1 gene (chromosome 22qter) have been shown to cause megalencephalic leukoencephalopathy with subcortical cysts, although there are patients in whom a mutation of MLC1 cannot be found. Recently, reports have appeared that describe a nonprogressive condition characterized by severe psychomotor delay with variable degrees of tone and reflex abnormality and MRI abnormalities consisting of Bilateral Anterior Temporal Lobe Cystic Lesions with pericystic abnormal myelination and symmetric patchy lesions with increased signal in the frontal and occipital periventricular white-matter regions (Henneke etb al., 2005). The clinical and neuroimaging features of leukoencephalopathy with bilateral temporal lobe cysts are distinct from those of megalencephalic leukoencephalopathy with subcortical cysts. Patients with leukoencephalopathy with bilateral anterior temporal lobe cysts are severely involved from the start and have a nonprogressive course, normal or small heads, and unique MRI findings. Another progressive condition that associates a leukoencephalopathy, and parenchimal cysts was descibed in 1996 by Labrune et al. as a Leukodystrophy, with formation of Parenchymal Cysts and evidence of Diffuse Cerebral Microangiopathy. Consanguinity in parents of one patient and occurrence in siblings suggests autosomal recessive inheritance. Finally a recent condition has been reported by Naidu et al. (2005) defiend as Progressive Cavitating Leukoencephalopathy characterized by episodic acute onset of irritability or neurological deficits that start between 2 months and 3.5 years of age, followed by steady or intermittent clinical deterioration. Brain MRI shows patchy leukoencephalopathy with cavities, and vascular permeability, in actively affected regions. Early lesions affect corpus callosum and centrum semiovale, with or without cerebellar or cord involvement. After repeated episodes, areas of tissue become larger cystic regions in brain or spinal cord. Elevated levels of lactate in brain, blood, and cerebrospinal fluid, abnormal urine organic acids, and changes in muscle respiratory chain enzymes are present but inconsistent. Familial occurrence and consanguinity suggest autosomal recessive inheritance of this distinct entity.

MLC1 LEUKOENCEPHALOPATHY

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Megalencephalic leukoencephalopathy with subcortical cysts (MLC) is a rare inherited, autosomal recessive form of childhood-onset spongiform leukodystrophy characterized by white matter abnormalities with vacuolation of the outer myelin sheaths. Mutations in a gene named MLC1 have been linked to MLC disease. Due to the abundant expression of MLC1 protein in astrocytes, it has been hypothesized that this disease may result from a primary astrocytic defect. However, the pathophysiological role of MLC1 is still unknown. To clarify this issue, an affinity purified rabbit polyclonal antibody against the whole recombinant human MLC1 protein was generated and used to study the expression, localization and biochemical properties of endogenous MLC1 in astrocytes in vivo and in vitro. Immunostaining of normal human brain tissue confirmed the predominant MLC1 localization in astrocyte end-feet in perivascular, periventricular and subpial regions. RT-PCR and western blot analyses indicated that endogenous MLC1 is abundantly expressed in cultured rat astrocytes and also in normal and tumoral human astrocytes. Our MLC1 antibody stained a protein of 36 kDa, which was detected in both the cytosolic and membrane fractions of the astrocytic extracts. A second protein of 60-65 kDa, probably a dimeric MLC1 complex, was also detected in the astrocyte membranes and Wheat germ agglutinin-enriched extracts. The identity of the above bands was confirmed by MALDI-TOF analysis. In vitro and in vivo double immunostainings with anti-MLC1 and anti-dystroglycan antibodies revealed a colocalization of the two molecules in the astrocyte end-feet. Dystroglycan is the central component of the dystrophin-associated protein (DAP) complex, which in astrocytes is involved in cytoskeleton-extracellular matrix interactions and anchoring of ion and water channels to the membrane. Interestingly, mutations in some DAP component genes have been reported to be associated with white matter abnormalities. To elucidate the MLC1 function we are currently investigating MLC1 molecular interactions performing immunoprecipitation experiments in combination with MALDI-TOF analysis.

SESSION 4-2 : GENETICS AND THERAPEUTICS OF LEUKODYSTROPHIES

THERAPEUTIC APPROACHES IN LEUKODYSTROPHIES

Chair
Odile BOESPFLUG-TANGUY (Inserm U.384, Clermond-Ferrand-France)

INTRODUCTION

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SCREENING COMPOUNDS TO AMELIORATE EIF2B DEFICIENCY

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eIF2B-related disorders (eRD) are a group of inherited autosomal-recessive fatal brain diseases mainly affecting children. There is currently no treatment or therapy available. The disorders primarily affect glial cells of the brain leading to progressive destruction of the white matter. The diseases are caused by mutations in a highly conserved protein synthesis factor called eukaryotic initiation factor 2B (eIF2B). eIF2B is a guanine nucleotide exchange factor for its G-protein partner called eIF2, and recycles eIF2 from an inactive GDP-bound to an active GTP-bound form. eIF2•GTP binds initiator-methionyl-tRNA (Met-tRNA$_i^{Met}$) [the tRNA required to initiate synthesis of all proteins]. eIF2B therefore functions in a basic cell process that is fundamental to all forms of life. It was highly unexpected therefore that mutation of a protein synthesis factor whose function is essential in all cells should cause this largely tissue-specific disease.

In my laboratory we have been studying the structure-function and regulation of eIF2B in translation and its control using the model eukaryote yeast *Saccharomyces cerevisiae*. eIF2B is a large multisubunit protein and our work has helped define how eIF2B functions and how it mediates cell responses to environmental stresses. Because of the structural and functional homology between human and yeast eIF2B our findings are directly relevant to the function of human eIF2B. To directly assess how human mutations causing eRD affect eIF2B function we previously introduced changes equivalent to human eIF2B mutations to subunits of yeast eIF2B. Analysis of the mutated yeast cells identified phenotypes indicative of a partial defect in eIF2B activity and responses to stresses that target eIF2B. These findings correlated well with subsequent studies by others with samples from eRD patients, thereby confirming the utility of the yeast model.

As eRD are recessive and mutated eIF2B retains some function, in theory any compound able to enhance or repair the remaining eIF2B function should help slow or prevent further disease progression. Our current aim is therefore to screen libraries for a candidate compound that could be developed into a therapeutic agent to treat eRD patients.

In order to perform compound screens, we have developed two assays based on our findings with yeast eRD mutations. The primary assay monitors cell growth. eIF2B mutated yeast cells have a translation defect so grow slower than wild type cells. Any compound that corrects the eIF2B defect should therefore repair the growth defect. The second assay monitors *GCN4* expression. *GCN4* is a stress-responsive gene translationally-controlled and hypersensitive to eIF2B activity. An equivalent human gene is called *ATF4*. eIF2B-mutated yeast cells have aberrantly high *GCN4* expression and this can be monitored quantitatively using a reporter-gene (b-galactosidase). As traditional b-gal substrates are not suitable for high-throughput screening in a miniaturized multiwell format, we have adapted and optimized a system marketed by Promega Corp. (devised for mammalian cell assays) for use with yeast cells. We have screened a library of 2000 compounds ‘in house’ and are initiating a robotic screen of ~70,000 compounds in conjunction with the NIH molecular libraries initiative screening

centre at the University of Pennsylvania. My presentation will highlight progress to date and planned future developments of this system.

GENE AND CELL THERAPY IN LEUKODYSTROPHIES

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The use of cell therapy and viral vectors to deliver genes to the nervous system show great promise for therapeutic applications in several neurodegerative diseases of the CNS, including diseases of white matter like leukodystrophies. The methodologies are continually undergoing refinement and for each application, a number of factors must be considered. These include the efficiency with which CNS target cells must be corrected, the number and type of CNS cells that must corrected to achieve intended changes, whether regulation of the transgene is required, and the level of toxicity that can be tolerated. Cerebral (and even worst, spinal cord) white matter presents a challenging target for gene delivery because most viral vectors with potential use in clinics (adeno-associated virus, lentiviral vector) do not transduce oligodendrocytes in vivo. Generally, diffusion of vector is limited from injection sites and transduction restricted to neurons and few astrocytes. However, one may take advantage that the therapeutic protein can be secreted by neurons and recaptured at distance by oligodendrocytes, as recently shown in animal models of metachromatic leukodystrophy (MLD) and Krabbe disease which are due to the defiency of a lysosomal enzyme. Neuronal cell bodies some distance from the injected area can also be transduced at some extent by anterograde or retrograde transport of adeno-associated virus and lentiviral vectors within their process. The use of siRNA has opened the possibility to down-regulate genes, as in Pelizaeus-Merzbacher disease due to duplication of the PLP gene. In newborn animals, delivery of secreted protein or enzyme might also be achieved using migratory embryonic stem cells, neural or oligodendrogial precursor cells that can also be engineered to release the enzyme. Encouraging results have been obtained in neonatal MLD mice. The embryonic or newborn brain is more permissive to vector diffusion and cell migration, but also more sensitive to damage than the adult brain. Peripheral nerve demyelination is also present in several leukodystrophies, requiring additional therapeutic approaches that may include enzyme replacement therapy. In short term, ex vivo gene therapy using hematopoietic stem cells engineered with lentiviral vectors may prove to be successful in adrenoleukodystrophy and MLD, two leukodystrophies in which allogeneic hematopoietic stem cell transplantation has clear clinical benefit, even it is only in selected patients and during a narrow therapeutic window.

COMBINED THERAPIES FOR THE TREATMENT OF KRABBE DISEASE.

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Galactocerebrosidase (GALC) is the enzyme responsible for the lysosomal catabolism of galactosylceramide and psychosine. The deficiency of GALC activity results in the severe autosomal recessive disorder called globoid cell leukodystrophy (GLD) or Krabbe disease. The accumulation of psychosine results in the apoptotic death of oligodendrocytes and Schwann cells. Other mechanisms including activation of resident microglial cells, recruitment of blood macrophages and expression of pro-inflammatory cytokines also play important roles in the pathogenesis. The only treatment currently available for some human patients is hematopoietic stem cell transplantation. There are several mouse models with low GALC activity available for study. Bone marrow transplantation of young affected mice can prolong their lives to over one year. The brains of long-lived mice had psychosine levels near normal, low, but significant, GALC activity and evidence for remyelination. The PNS also shows impro vement but will require additional treatment. GALC cDNA has been cloned into several AAV serotypes. When these viral vectors were injected intra-ventricularly or intra-cranially into the brains of newborn affected mice, frozen sections stained with both anti-GALC antibodies and with X-gal. Injected mice lived up to 80 days, despite the high expression of GALC activity in brain. There was improved myelination and a reduction in the psychosine concentration. In an attempt to treat the PNS, the AAV2/1 vector was injected into leg muscles, and high GALC expression was obtained. It seems apparent that more than one approach may be needed to prevent and correct the pathology seen in both the CNS and PNS in this disease.

INHIBITING PLP GENE MUTATIONS

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Mutations in the PLP/DM20 gene in humans are associated with a spectrum of X-linked disorders spanning in clinical severity from PMD to SPG2. Approximately 60% of patients with classical PMD carry duplications of the PLP/DM20 gene, suggesting a gene dosage mechanism. Transgenic mice carrying extra copies of the PLP gene develop neurological deficits, the severity of which correlates with PLP copy number, and have abnormal accumulation of cholesterol with PLP in late endosomes of oligodendrocytes (OLs). Point mutations in the PLP/DM20 gene cause both severe and milder forms of PMD and SPG2. In the jimpy (*jp*) mouse, a single base change causes excision of exon 5, a frameshift, and complete alteration of the 5' end of the predicted protein. *Jp* mice show severe dysmyelination and premature death. *Jp* OLs have numerous defects which are likely caused by the combined loss of normal PLP expression and production of abnormal *jp* PLP. Both PLP over-expressor and *jp* mice are models of PLP disorders in humans.

In this work, we sought to correct the cellular defects and restore a normal phenotype by reducing PLP over-expression and silencing *jp* PLP with shRNA that target PLP/DM20 in over-expressor and *jp* OLs. We have assessed reduction of PLP over-expression and reversal of cellular abnormalities in cholesterol metabolism in Oli-neu cells transfected with PLP-Myc. The accumulation of cholesterol in late endosomes (LE) induced by PLP-Myc over-expression was measured by counting the number of cells in which filipin and LAMP1 are co-localized in transfected vs. untransfected Oli-neu cells. Cholesterol is present in LE in 66$\pm$2.5% of transfected Oli-neu cells vs. 24$\pm$2% of untransfected Oli-neu. Oli-neu cells transfected with Myc-PLP were treated with a cocktail of siRNA that targets the first 200 nucleotides of the PLP transcript, previously shown to reduce PLP expression by 60% in primary OL. After 48 hrs in differentiation medium, the number of cells in which filipin and LAMP1 were colocalized was 36$\pm$1% in siRNA treated cells vs. 66$\pm$2.5% in untreated cells. The reduction in PLP transcript detected by RT-PCR after treatment with siRNA targeting PLP vs. mock treated cells was of similar degree as the reduction in filipin and LAMP1 co-localization. These data show that treatment with siRNA causes a statistically significant decrease in the accumulation of cholesterol in late endosomes in cells over-expressing PLP. We have assessed whether silencing *jp* PLP normalizes the phenotype and function in 158JP cells, an immortalized cell line derived from primary *jp* OLs that mimic phenotypic alterations in primary *jp* OLs, including elevated $[Ca^{++}]_i$, lack of maturational Em hyperpolarization, minimal production of myelin proteins, and abnormal responses to dibutyryl cAMP. Both transient and stable transfection experiments showed that siRNA constructs targeting *jp* PLP gene silenced *jp* PLP. We show that silencing *jp* PLP corrects the abnormal $[Ca^{++}]_i$ in 158JP cells as determined by Fura2 ratios, and permits 158JP cells to undergo the same maturational hyperpolarization that occurs in normal OLs. Normal OLs differentiate in response to dbcAMP and show strong expression of inducible cAMP early repressor (ICER) mRNA, while *jp* OLs do not. Northern blot analysis showed that silencing *jp* PLP restores dbcAMP induced ICER expression in 158JP cells. Finally, we detected a partial restoration in levels of CNPase and the presence of the CNPase doublet in silenced

158JP cells. These studies demonstrate that reduction of *jp* PLP gene expression corrects a number of defects that occur in both 158JP and *jp* OLs. However, full restoration of a normal phenotype in *jp* OLs might additionally require the presence of normal PLP.

Studies are underway to silence PLP or *jp* PLP in dissociated and cerebellar slice cultures prepared from the PLP over-expressor and *jp* mice, to demonstrate reversal of the cellular abnormalities described above, and assess OL survival, lineage progression and restoration of myelin production. Future studies will begin to translate these results in vivo, using intrathecal infusions of either siRNA or antisense morpholino compounds targeting PLP and *jp* PLP to improve the mutant phenotype.

CONCLUDING

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POSTERS

MOLECULAR MECHANISMS OF OLIGODENDROGLIAL SPECIFICATION MEDIATED BY SHH

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In the embryonic chick ventral spinal cord, the initial emergence of oligodendrocytes is a relatively late event that depends on prolonged Sonic hedgehog (Shh) signaling. In this report, we show that specification of oligodendrocyte precursors (OLPs) from ventral Nkx2.2-expressing neural progenitors occurs precisely when these progenitors stop generating neurons, indicating that mechanism of neuronal/oligodendroglial switch is a common feature of ventral OLP specification. We further show that an experimental early increase in the concentration of Shh is sufficient to induce premature specification of OLPs at the expense of neuronal genesis indicating that the relative doses of Shh received by ventral progenitors determine whether they become neurons or glia. Accordingly, we observe that the Shh protein accumulates at the apical surface of Nkx2.2-expressing cells just prior to OLP specification, providing direct evidence that these cells are subjected to a higher concentratio n of the morphogen when they switch to an oligodendroglial fate. Finally, we show that this abrupt change in Shh distribution is most likely due to the timely activity of Sulfatase 1 (Sulf1), a secreted enzyme that modulates the sulfation state of HSPGs. Sulf1 is expressed in the ventral neuroepithelium just prior to OLP specification and we show that its experimental overexpression leads to apical concentration of Shh on neuroepithelial cells, a decisive event for the switch of ventral neural progenitors towards an oligodendroglial fate.

A HIGH THROUGHPUT SCREEN FOR THERAPEUTIC COMPOUNDS TO AMELIORATE eIF2B-RELATED DISORDERS

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eIF2B-related disorders (eRD) are a group of inherited autosomal-recessive brain diseases mainly affecting children. There is currently no treatment or therapy available. The disorder primarily affects glial cells of the brain with destruction of the white matter. The diseases are caused by mutations in eukaryotic initiation factor 2B (eIF2B). eIF2B is a guanine nucleotide exchange factor for eIF2, that recycles eIF2 from a GDP to an active GTP bound form, this process is necessary to form the ternary complex that is required for protein translation initiation in cells. Because of the structural and functional homology between human and yeast eIF2B, we previously used yeast (*Saccharomyces cerevisiae*) to study this disorder and introduced changes equivalent to human eIF2B mutations to subunits of eIF2B. The yeast mutated cells had phenotypes indicative of defects in eIF2B activity, which correlated with subsequent studies of others with samples from eRD patients.

Our aim is to find a compound that could be used as a therapeutic agent to treat these patients. In order to perform a drug screen, we have used two assays. The primary assay monitors cell growth. eIF2B mutated yeast cells grow slower than wild type cells, so any compound that corrects the eIF2B defect should repair the growth defect. The secondary assay monitors yeast *GCN4* expression. *GCN4* gene encodes a transcription factor that is responsible for the activation of the expression of several genes of the amino acid biosynthesis, under stress (for example starvation) situation that phosporylate eIF2 the global translation is reduced, but expression of *GCN4* is enhanced. In the yeast cells used in our study, *GCN4* was fused with beta-galactosidase. In eIF2B mutated yeast cells the translation of *GCN4* is enhanced compared to the yeast wild type, so any compound that reduces the *GCN4* levels close to the wild type would be a possible compound to be used to treat eRD patients. To perform this secondary assay, we have adapted a system from Promega devised for mammalian cells to be used with yeast cells. The two step assay consists of mixing the cells with a buffer containing beta-galactosidase substrate (6-O-β-galactopyranosil-luciferin) that is cleaved by beta-galactosidase present in the cells, releasing D-Luciferin. This compound is a substrate for firefly luciferase that produces light that is quantitated using a luminometer. The main advantages of this system are sensitivity and that no centrifugation steps are required. In our laboratory, we have recently undertaken a screen with 2000 compounds library and we are currently starting a robotic screen of 63,000 compounds in collaboration with the NIH Molecular Libraries Initiative at the University of Pennsylvania.

TRACT-BASED SPATIAL STATISTICS (TBSS) ANALYSIS OF DIFFUSION TENSOR IMAGING DATA REVEALS DECREASED FRACTIONAL ANISOTROPY IN WHITE MATTER IN PRETERM INFANTS IMAGED AT TERM EQUIVALENT AGE

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Diffusion tensor imaging (DTI) quantifies the diffusion of water within tissues and is a non-invasive, non-ionising method of evaluating brain microstructure in vivo. We performed DTI on a group of preterm infants imaged at term equivalent age that had no evidence of abnormality on conventional magnetic resonance imaging (MRI) (n = 26). We compared their diffusion parameters to postmenstrual age- and sex-matched term-born controls (n = 6) using an automated tract-based approach (TBSS)1 to test the hypothesis that fractional anisotropy is reduced in white matter regions in the preterm brain compared to term born control infants. We found that the centrum semiovale, frontal white matter, external capsule, posterior limb of the internal capsule and genu, splenium and isthmus of the corpus callosum had a significantly lower fractional anisotropy (FA) compared to term-born controls. Those infants born before 28 weeks gestational age (n = 11) displayed additional reductions in FA in the middle portion of the body of the corpus callosum and within the optic radiations. To further characterise this reduction in FA, we analysed the three independent eigenvalues of the diffusion tensors and found that the reduction in FA values observed in the preterm infants was due to an elevation in the intermediate (É…2) and minor (É…3) eigenvalues. In conclusion, this automated tract-based approach is able to identify white matter abnormalities in the preterm brain in the absence of focal lesions and obviates the need for observer-dependent region of interest analyses.

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RHIZOMELIC CHONDRODYSPLASIA PUNCTATA AND THE ROLE OF PLASMALOGENS IN ITS PATHOGENESIS, INCLUDING THE ABNORMAL MYELIN FORMATION.

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Background: Rhizomelic chondrodysplasia punctata (RCDP) is an autosomal recessive peroxisomal disorder, clinically characterized by typical facial appearance, congenital cataracts, skeletal abnormalities, and severe psychomotor retardation. Milder phenotypes exist. The main biochemical abnormality in RCDP is a defect in plasmalogen biosynthesis, due to either a misdirecting of three peroxisomal enzymes to the peroxisome (RCDP type 1) leading to a deficiency of plasmalogens and accumulation of phytanic acid, or a defect in one of the peroxisomal enzymes involved in the plasmalogen biosynthesis (RCDP type 2 and 3).
Since plasmalogen deficiency is present in all three subtypes and the phenotypes are clinically indistinguishable, plasmalogens are thought to be an important factor in the pathogenesis of the disorder, which is also reflected in the MRI patterns of patients. Patients with the milder clinical phenotype of RCDP and a significant residual capacity to synthesize plasmalogens have no abnormalities on MRI, whereas patients with the severe phenotype show myelin abnormalities and progressive cerebellar atrophy.
Aims: Evaluation of batyl alcohol supplementation as a therapeutic agent for RCDP patients.
Methods: Patients with biochemically proven RCDP in The Netherlands are eligible to enter this cohort study. Patients will be taking 5 to 50 mg/kg batyl alcohol daily, which will be administrated orally. The duration of the study is 1 year. The primary endpoint in this study is the plasmalogen content of erythrocytes.
Results: After 6 months treatment all patients are at 50 mg/kg/day and show a 3-fold increase of plasmalogens, without any side effects. Continuous evaluation will determine if this treatment results in clinical improvement in patients with RCDP.

INTRATHECAL CHRONIC INJECTIONS OF A POTENTIALLY NEUROPROTECTIVE AGENT IN THE MOUSE: FEASABILITY, ABSENCE OF TOXICITY AND MRI INTERFERENCES

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Abstract

A new surgical procedure was developed in order to test the potential therapeutic effect of a neuro-protecting (or/and neuro-repairing) molecule. The procedure consisted in injecting a neuroactive peptide (NS) deduced from the SCO-spondin protein in the intratechal space of mouse spinal cord. This peptide was previously evaluated on animal models of spinal cord injuries using a unique administration at the lesion site. A regrowth of nerve fibers and an improvment of the mice motor performances were observed in the days following the injection. In the aim to test the potential therapeutic interest of NS on the axonopathie observed in *plp1*Y mice, we developped a new protocol using inthrathecal chronic injections.
The mouse was anesthetized by isoflurane and placed in a prone position on a heating cover under a binocular microscope. The T11/T12 vertebrae being exposed, the catheter was carefully inserted in the subarachnoid space through the pia matter. The catheter was then pushed within the subarachnoid compartment in the direction of the brain and was sticked on T12 lamina. After suture, the catheter entry appeared near the occiput. This procedure was well tolerated by the mice and allowed weekly injections of the drug.
The peptide, resuspended in sterile water, was delivered at a dose of 37.5 µg/kg using a Hamilton syringe. The administration was done through the intrathecal catheter previously implanted. A fresh solution of peptide was prepared once every 15 days and then used for two injections. This aqueous solution was conserved at +4°C between two experiments.
Following the catheter implantation, a toxicity study was performed on nine C57 BL6 wild type mice. Treated mice received weekly injections of the peptide during 1 to 5 months whereas control mice received the vehicle. After each injection, animals were placed in individual cages with food and water *ad libitum*. Animals were regularly observed for behavioural or physical changes. They were weighed every two days in order to control their growth, and a locomotor evaluation was conducted using rotarod test.
Our results indicates that both the implantation of the catheter in the spinal cord subarachnoid space of the mice and the chronic injections of the peptide did not affect the growth or the motor performances of the mice. In addittion, presence of the catheter did not change the in vivo MRI conditions we have developped for the longitudinal follow up of mouse spinal cord (see poster Ben Hassen W et al)
This workdemonstrates the feasability of longitudinal studies using weekly injections of this peptideto evaluate by magnetic resonance imaging *in vivo*.its preventive and curative effect on the neurodegenerescence observed in dysmyélinating mutant mice.

IN VIVO MAGNETIC RESONANCE IMAGING OF THE MOUSE SPINAL CORD FOR THE LONGITUDINAL STUDY OF AXONOPATHIE AND DYSMYELINATION

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Axonopathy of KO-PLP mouse has rostro-caudal progression. Therefore this disease affects spinal cord (SC) tracts with an ascending progression from the lombo-sacral to the cervical regions and finally to the brain. Moreover, comparing to the brain, the important volume occupied by the white matter (WM) in the SC and the well-organized tracts oriented in the rostro-caudal direction are important practical advantages. Hence NMR techniques are suitable to image the SC in order to early assess the axonopathy development and during any therapy.

Magnetic resonance imaging (MRI) at high field provides non invasive tools to examine *in vivo* the central nervous system (CNS) of rodent, and then to monitor microstructural changes within a longitudinal study. A variety of established MRI sequences provides anatomical and/or functional information about biological tissues. Among them, diffusion-weighted MRI (DWI) is a method of choice for evaluating the integrity of white matter tracts. DWI measures the apparent diffusion motion of water molecules, thereby providing microstructural information beyond the spatial resolution of MRI. The diffusion of water in the CNS is known to be affected by various microscopic barriers e.g cell membranes and myelin sheaths, and by their organisation. Therefore, DWI may specifically characterize axono- and myelino-pathies.

Specific diffusion MRI methods have been developed for this purpose at high field; PGSE (Pulsed Gradient Spin Echo) and LSDI (line scan diffusion imaging). These techniques require respiratory gating to minimize motion-related artefacts. Animals are then intubated, curarized and mechanically ventilated using a MRI-compatible rodent ventilator. Curarization is important for maintaining a steady breathing without any resistance of respiratory muscles during experience. The principal advantages of LSDI sequence compared to PGSE are the possibility to observe a limited region of SC and the reduced sensitivity to motion. We present here DWI obtained on living mice demonstrating the efficiency of these two complementary approaches for high resolution imaging of SC.

The LSDI sequence will be used to characterize the dysmyelinopathy of the PLP over expressing mice and the axonopathy of the KOPLP mice. Besides PGSE sequence will be applied to follow up a repairing and/or preventive therapy on the KOPLP mice using a neuro-active peptide (NS).

PHARMACOLOGICAL TREATMENT BASED ON GENE REDUNDANCY: A NOVEL THERAPEUTIC APPROACH FOR X-LINKED ADRENOLEUKODYSTROPHY

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X-linked adrenoleukodystrophy (X-ALD) is a severe neurodegenerative disorder comprising widely different clinical phenotypes all caused by mutations in the ABCD1 (adrenoleukodystrophy) gene. Overexpression of ABCD2 (adrenoleukodystrophy-related), the closest relative of ABCD1, restores the biochemical defect in cultured fibroblasts of X-linked adrenoleukodystrophy patients as well as the clinical symptoms in the Abcd1-deficient mice. The different expression patterns of human ABCD1 and ABCD2 could be reason why ABCD2 is not able to compensate for ABCD1 deficiency in X-linked adrenoleukodystrophy patients. Pharmacological induction of ABCD2 expression in disease-relevant cell types has been targeted as a novel therapeutic approach for X-linked adrenoleukodystrophy. Cholesterol lowering activates human ABCD2 in cultured cells. A functional sterol regulatory element (SRE) was identified in the human and murine ABCD2 promoter overlapping with a functional DR4 regulatory element t hat is able to bind nuclear receptors liver X receptor alpha/retinoid X receptor alpha heterodimer as well as thyroid hormone receptor beta/retinoid X receptor alpha but not liver X receptor alpha and sterol regulatory element-binding protein at the same time. In vitro liver X receptor agonists inhibit sterol regulatory element-binding protein-mediated induction of ABCD2. Thus, agonists and antagonists of nuclear hormone receptors seem to be promising candidates for therapeutic modulation of ABCD2 gene expression. Supported by: The Myelin Project, ELA and EU

$GABA_A$ RECEPTOR SUBUNIT EXPRESSION IN OLIGODENDROCYTES OF RAT CEREBELLAR SLICES.

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Oligodendrocytes are damaged in conditions like perinatal asphyxia/cerebral palsy, stroke, spinal cord injury and multiple sclerosis, in part by activation of glutamate receptors. To examine the potential physiological and pathological role of receptors in oligodendrocytes for the inhibitory amino acid GABA, we whole-cell clamped oligodendrocytes in the white matter of cerebellar slices (Káradóttir et al., 2005). Cells at different developmental stages were identified via their morphology (correlated with labelling for NG2, O4 and MBP in some cells). At all stages oligodendrocytes showed a current in response to 100μM GABA, mediated by $GABA_A$ receptors, which was blocked by bicuculline, while a $GABA_B$ receptor agonist and antagonist, a $GABA_C$ antagonist, and the GABA transporter (GAT-1) blocker SKF89976A, had no effect on the baseline or GABA-evoked current. In mature oligodendrocytes the current generated was inward even when the membrane potential was clamped positive to the value of E_{Cl} for the pipette solution, suggesting poor clamping of the voltage and/or $[Cl^-]_i$ in the long and thin myelinating processes (or possibly that GABA also blocks a K^+ current: Pastor et al., 1995).

To examine the $GABA_A$ receptor subunits that might generate these currents, we first carried out light microscopic immunocytochemistry on cerebellar white matter. Labelling was observed for the both the α_1 and α_2 subunits, where α_1 antibody labelled both processes aligned with axons and the membranes of the cell bodies, but the α_2 antibody labelled the processes more than the cell bodies. Omitting the primary antibody abolished the labelling. When looking at β subunit expression, only antibody to β_1 gave strong staining and, like the α_2 antibody, it preferentially labelled the processes relative to the cell bodies. Further, β_3 antibody labelled some of the processes in the white matter but not as strongly as β_1 antibody. Antibody to β_2 subunit did not label the white matter at all, although it did label the granule cell layer. Finally, antibody to the γ_2 subunit also labelled the white matter, primarily the cell processes.

To investigate the subcellular localization of $GABA_A$ receptors in more detail we used post-embedding immunogold electron microscopy on adult rat cerebellum (Landsend et al., 1997; Káradóttir et al., 2005). The γ_2 subunit was present in the myelinating processes of oligodendrocytes, in the outer and innermost membranes of the myelin sheath and also within the myelin. By quantifying the immunogold particles in the myelin in white matter, in myelin in the granule cell layer, and at the symmetrical Purkinje cell dendritic synapses (inhibitory synapses), we found that the mean density of γ_2 subunit throughout the myelin in the white matter and in the granule cell layer was 57% and 63% of that in the postsynaptic density of the inhibitory synapses onto Purkinje cells.

From these data, it is likely that the oligodendrocyte $GABA_A$ receptors include α_1 and/or α_2, β_1 and γ_2 subunits in their composition. Release of GABA (Shimada et al., 1993) might contribute to oligodendrocyte depolarization during conditions of energy deprivation. The

presence of $GABA_A$ receptors in the myelin might explain why the anti-epileptic drug vigabatrin, which raises extracellular GABA levels by inhibiting the GABA degrading enzyme GABA transaminase, can cause swelling and loss of myelin (Sidhu et al., 1997)

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MLC DISEASE: ELUCIDATING THE ROLE OF MLC1 PROTEIN IN BRAIN PHYSIOLOGY AND PHYSIOPATHOGENESIS; RESEARCH FOR MLC GENES.

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The syndrome of megalencephalic leukoencephalopathy with subcortical cysts (MLC, MIM 604004) is a rare vacuolating myelinopathy characterized by early onset progressive ataxia, and pyramidal tract involvement initially confined to lower limbs with subsequent diffusion to upper limbs. The distinctive features are megalencephaly - which starts in the first year of life ˆ and diffuse white matter abnormalities with subcortical cysts in the tips of the temporal lobes and in frontoparietal subcortical areas. Contrary to other leukoencephalopathies, MLC has a relatively milder clinical evolution. Genetically, MLC is a heterogeneous condition. A first locus (MLC1) was mapped on chromosome 22q13.33 in Turkish families. A total of 28 MLC cases have been presently analyzed for MLC1 gene : 19 patients presented mutations. In this preliminary analysis, we confirmed previous evidence that MLC patients show variability in severity of clinical symptoms and disease progression. So far, no genotypeˆphenotype correlation has been found. To identify the second locus linked to MLC1 disease, we performed a genome-wide SNP distribution analysis on three MLC patients from Umbria, excluded for MLC1 locus, with the hypothesis of a common ancestor because of a shared origin from a relatively restricted area. After, linkage analysis on Genehunter software, we detected an homozygote region on chromosome 2q35-q36.1. We sequenced genes or transcripts resulting from more deep in silico analysis of the region of interest, no mutation was found. The region of interest on ch2q35-q36.1 was also analyzed and excluded in an additional family using microsatellites analysis, suggesting the existence of at least 3 MLC loci.

HYPOMYELINATION AND CONGENITAL CATARACT (HCC): A NOVEL WHITE MATTER DISORDER DUE TO THE DEFICIENCY OF HYCCIN A NEWLY IDENTIFIED MEMBRANE PROTEIN

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The systematic analysis of cases with unclassified leukoencephalopathies allowed us to identify 10 patients showing congenital cataract, slowly progressive neurological impairment, and diffuse white matter abnormalities on MRI. All patients presented with congenital cataract. Psychomotor developmental delay was evident after the first year of life. All cases achieved the ability of walking with support only, and they lost this ability with time. Neurological examination showed pyramidal and cerebellar signs. Mental retardation ranged from mild to moderate. Peripheral neuropathy was demonstrated by neurophysiological studies in 9/10. MRI showed global involvement of the supratentorial white matter, with preservation of both cortical and deep gray matter structures. There was no abnormal contrast enhancement. Sural nerve biopsy revealed deficiency and disorganization of the myelin sheath in several nerve fibers. Linkage studies led to the identification of a 3.2 Mb homozygous segment on chromosome 7p21.3-p15.3 in all affected patients. Three different mutations were identified in a gene of unknown function *(DRCTNNB1A)* encoding a 521aa protein (hyccin) we demonstrated to be tightly associated to the membrane protein. Furthermore, we showed that the mutations lead to complete or severe hyccin deficiency. In conclusion**,** we have identified a novel autosomal recessive white-matter disorder characterized by hypomyelination of the central and peripheral nervous system, progressive neurological impairment, and congenital cataract (Hypomyelination and Congenital Cataract, HCC). A novel membrane protein, hyccin, is responsible for HCC and plays an essential role in central and peripheral myelination.

OPCs DIFFERENTIATION IN THE CEREBELLUM IS CONTROLLED BY A NOVEL DIFFUSIBLE FACTOR

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Proliferation and differentiation of the oligodendrocyte precursor cells (OPCs) are thought to be regulated by the finely tuned balance between intrinsic properties and factors of the environment. To understand how the extrinsic factors modulate the OPC differentiation, we performed experiments with cerebellar organotypic cultures. We have shown that when newborn (P0) cerebellar (receiver) slices were co-cultured - at a distance - with other P0 (donor) slices already kept in vitro for 7 days (P0 + 7DIV), the OPC differentiation in the receiver slices was greatly enhanced during the first 7DIV. The oligodendrocyte differentiating factor is present in the conditioned medium (CM) taken from donor slices between the 7th and 14th DIV. This oligodendrocyte differentiating factor is hence diffusible and age dependent (OADDF). To identify the cells producing OADDF, we used as donor slices P0 cerebellar explants treated during the first 3DIV with high concentration of 5-bromo-2-deoxyuridine (BT3-donor slices). As previously reported, in the P0-BT3 slices there is a depletion of oligodendrocytes and an increase in Purkinje cell survival. The CM of the P0-BT3 cultures exerted a more powerful OPC differentiation effect than the CM collected from untreated donor slices. In contrast, when the CM was collected from donor slices depleted in Purkinje cells by axotomy, the enhancement of OPC differentiation was abolished. These experiments show that Purkinje cells and not oligodendrocytes are the cell producing OADDF. Therefore, we have compared the differentiating action of OADDF w ith that of already known OPC differentiating factors produced by Purkinje cells such as IGF-1, TGFß and Progesterone. None of these factors mimic the effect of OADDF. Thus, further experiments are required to characterize this factor.

ROLE OF PHYTANIC ACID IN NERVOUS SYSTEM PATHOGENESIS USING MOUSE MODELS FOR REFSUM DISEASE

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Refsum disease is an autosomal recessive disorder clinically characterized by retinitis pigmentosa, peripheral polyneuropathy and cerebellar ataxia. Refsum disease is caused by mutations in the gene encoding phytanoyl-CoA hydroxylase (PHYH), the first enzyme of the peroxisomal alpha-oxidation pathway. Due to this deficiency the branched-chain fatty acid phytanic acid accumulates in plasma and tissues of Refsum patients. The aim of this project is to obtain insight into the patho-physiological consequences of an accumulation of phytanic acid in nervous tissue and to study the underlying mechanism using the PHYH knockout mouse and the PHYH:PPARalpha double knockout mouse as in vivo models. All experimental groups consisted of wild type mice, PPARalpha KO mice, PHYH KO mice and PHYH:PPARalpha DKO mice, and mice where fed a control diet (4 weeks) or a phytol diet (8 weeks) or combinations of the phytol diet followed by the control diet. At the end of the experiment the phenotype of the mice was evaluated using, the SHIRPA protocol, their locomotion using a CatWalk, neurophysiological analyses and, tissues were collected for biochemical and pathological analyses. Locomotion of PHYH KO mice after the phytol dietary periods was significantly affected and these mice also had slower motor nerve conductance velocities. Measurement of fatty acids in liver and total brain homogenates revealed an accumulation of phytanic acid in PHYH KO mice and in PHYH:PPARalpha DKO mice. Histological evaluation of brain tissue is underway and comparison between the different experimental groups will determine the pathological consequences of phytanic acid accumulation.

OLIGODENDROGENESIS FROM PRIMATE NEURAL STEM CELLS FOR MYELIN REPAIR OF THE DISEASED CENTRAL NERVOUS SYSTEM.

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The discovery of multipotent stem cells in the human adult central nervous system (CNS) has suggested novel potential therapies for myelin disorders including Pelizaeus-Merzbacher disease. Even though human neural stem cells (hNSC) can generate oligodendrocytes, the conditions required for optimal periventricular white matter regeneration by hNSC remain to be developed. Moreover, little is known about the factors that govern the differentiation of human and non-human primate neural stem cells into the oligodendroglial fate and as a consequence, the proportion of primate oligodendroglal cells generated remains very low. In order to derive cell populations enriched in oligodendrocytes and oligodendrocyte progenitors from early primate fetal brain (8-10 week post-gestation), we asked whether human and macaque forebrains contained discrete regions enriched in oligodendrocytes. Our in vitro data showed predominant oligodendroglial (A2B5+/GalC+) over neuronal (A2B5+/MAP5-) differentiation of the thalamus-derived cells compared to the cortex and ganglionic eminence. Second, we asked whether molecules known to be implicated in oligodendrocyte development can drive oligodendroglial differentiation from neural stem cells of these different regions in vitro. Our data show that the BMP inhibitor Noggin, Shh and anti-cystatin C which are known to induce oligodendrocyte differentiation in mouse NSC, were unable to elicit such fate in hNSC. To circumvent this liitation, oligodendrocyte progenitors can be selected on the basis of A2B5+/MAP5- expression. We are currently comparing the myelinating potential of hNSC from the different brain regions (total populations or enriched in oligodendrocyte progenitors) in vivo by grafting each of these populations into a model of dysmyelination, the shiverer mouse. This study should indicate which human foetal forebrain region would be the best source of myelin-forming cells and if a selection procedure could enhance this potential and therefore contribute to the development of potential therapies for myelin disorders. This work is funded by the ELA Foundation and INSERM.

GJA12 MUTATIONS IN AUTOSOMAL RECESSIVE HYPOMYELINATING LEUKOENCEPHALOPATHY

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Recently mutations in the gene *GJA12* encoding the gap junction connexin protein, connexin 47 (Cx47) have been associated with an autosomal recessive hypomyelinating leukoencephalopathy clinically characterized by nystagmus, cerebellar ataxia, and spasticity, fully resembling Pelizaeus Merzbacher disease. We screened for *GJA12* mutations in 10 families originating from Italy, Pakistan and Saudi Arabia and identified three novel homozygous mutations in 12 mutant cases distributed in 3/10 families. The mutations segregated with the disease according to an autosomal recessive trait, and included one missense (G236S) and two nonsense (L281fs285X and P131fs144X) changes. The identification of homozygous mutations predicting the synthesis of aberrant and truncated polypeptides clearly demonstrates that the loss of Cx47 function is the cause of the disease. The phenotype of *GJA12*-related leukoencephalopathy is fairly homogenous, and so similar to that of PMD to deserve the definition of Pelizaeus Merzbacher like disease (PMLD). However, slower progression of symptoms, greater preservation of cognitive functions, and partial myelination of cortico-spinal tracts at MRI are distinctive features, which could help in the differential diagnosis. Further studies are warranted to better delineate the phenotypic expression of *GJA12* mutations. Given the role of gap junctions in the intercellular communication in the nervous system, genes encoding connexin proteins expressed in glial cells may represent a new group of candidate genes for the still undefined leukoencephalopathies.

RUNDOWN AND INHIBITION OF OLIGODENDROCYTE NMDA RECEPTORS

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Pathologies such as cerebral palsy, spinal cord injury and multiple sclerosis involve glutamate-mediated damage to the white matter. We have recently shown that oligodendrocytes in the white matter of the cerebellum and corpus callosum express NMDA receptors, which are activated during ischaemic conditions (Káradóttir et al. 2005). Thus, as in neurons, these receptors may cause cell damage by glutamate-induced excitotoxicity. The aim of this study was to investigate further the properties of oligodendrocyte NMDA receptors, using whole-cell voltage-clamp recordings in rat brain slices.

Mature oligodendrocytes were whole-cell clamped with internal solution containing Cs^+ as the main cation and identified by their morphology as visualized by dye filling (Káradóttir et al. 2005). The peak current response evoked at -60mV by repetitive NMDA applications (60μM, once every ten minutes) exhibited a rundown, both in the corpus callosum and cerebellum. This rundown was area-dependent: in cerebellum the response to the third NMDA application was 33±5% (mean±s.e.m., n=13 cells) smaller than the first response, whereas in the corpus callosum it was 79±9% smaller (n=15; significantly different, p=0.0002 by 2-tailed t test). In neurons, NMDA response rundown can be reduced or abolished by lowering the extracellular calcium concentration (Rosenmund & Westbrook, 1993). However, in cerebellar oligodendrocytes, the rundown was larger when we decreased the extracellular calcium concentration from 2.5mM to 0.2mM (in 0.2mM $[Ca^{2+}]$ the response to the third NMDA application was 63±4% (n=8) smaller than the first one; significantly different to 2.5mM $[Ca^{2+}]$, p=0.0002 by 2-tailed t test).

NMDA receptor blockers have been widely tested as agents that may prevent grey matter damage in conditions like stroke. The discovery of NMDA receptors in oligodendrocytes (Káradóttir et al. 2005) suggests that these blockers may be useful for treating white matter diseases. Memantine, the only clinically-approved NMDA receptor antagonist, inhibited the NMDA-evoked current in corpus callosum oligodendrocytes (IC_{50}=30±12μM, 122 cells).

These data suggest that the NMDA receptors (or their downstream signalling) in oligodendrocytes differ from those in neurons and differ between white matter areas. Memantine or related compounds might be useful as a therapy for preventing white matter damage.

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siRNA TARGETING PLP CORRECT THE CELLULAR DEFECTS CAUSED BY PLP OVER-EXPRESSION

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Sixty percent of patients with PMD carry duplications of the PLP/DM20 gene, suggesting a gene dosage mechanism. Transgenic mice carrying extra copies of the PLP gene develop neurological deficits associated with abnormal accumulation of cholesterol and PLP in late endosomes (LE) of oligodendrocytes (OLs) (Simons et al. 2002). We sought to correct these cholesterol abnormalities by reducing PLP over-expression with siRNA in Oli-neu cells transfected with PLP-Myc. To quantitate cholesterol accumulation in LE, we counted the number of cells in which filipin and LAMP1 are co-localized in transfected Oli-neu cells. Cholesterol is present in LE in 66+2.5% of transfected Oli-neu cells vs. 24+2% of untransfected Oli-neu. Oli-neu cells transfected with Myc-PLP were treated with a cocktail of siRNA that targets 200 nucleotides of the PLP transcript, shown to reduce PLP expression by 60% in primary OLs. After 48 hrs in differentiation medium, filipin and LAMP1 were colocalized in 36+1% of siRNA treated cells vs. 66+2.5% of untreated cells. Reduction of PLP transcript in siRNA treated cells vs. mock treated cells was of similar degree as the reduction in filipin and LAMP1 co-localization. Thus, reduction of PLP by siRNA causes a decrease in the accumulation of cholesterol in LE in cells over-expressing PLP. Studies are underway to silence PLP in dissociated and cerebellar slice cultures prepared from the PLP over-expressor mice, and assess reversal of the cellular abnormalities, OL survival and restoration of myelin.

DETECTION OF NOVEL MUTATIONS IN ALEXANDER DISEASE USING DHPLC ANALYSIS OF GFAP GENE

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Alexander Disease (AXD) is a severe neurological disorder, characterized by a leukodystrophy associated with Rosenthal Fibers (RFs, eosinophilic inclusions within astrocytes). This autosomal dominant disease is caused by *de novo* mutations in Glial Fibrillary Acidic Protein (GFAP) gene. Mutations seem to induce a toxic gain of function resulting in GFAP aggregation in astrocytes (RFs) and subsequent white matter suffering.
In infantile and juvenile classical forms of AX, brain MRI criteria have been defined in order to screen patients for GFAP mutations. This molecular screening is usually performed by *GFAP* gene amplification and direct sequencing. However, numerous GFAP mutated patients with atypical brain imaging and unusual clinical symptoms have been described. To improve the rapidity of this screening for a larger number of patients, we developped a new protocol based on DHPLC analysis of PCR products and selective sequencing of abnormal profiles.
During the last 2 years, 67 patients (29 index cases, sporadic and some familial forms) were entirely tested by DHPLC. 20 index cases were found mutated with 13 different mutations, 8 of which being previously reported. DHPLC detected 5 novel mutations believed to generate AXD as: mutations arose *de novo*, were absent from healthy relatives and from 100 control chromosomes, affected a conserved amino-acid and a functionally important domain, and no other change was detected. Several prenatal diagnoses have been already carried out. DHPLC screening for *GFAP* mutations is confirmed to be a rapid, sensitive and reliable, cost-effective and high-throughput method for AXD diagnosis.

HEMATOPOIETIC CELL TRANSPLANTATION PREVENTS NEUROLOGICAL DISEASE IN ADRENOLEUKODYSTROPHY MOUSE

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X-linked adrenoleukodystrophy (ALD) is characterized by progressive demyelination and accumulation of very-long chain fatty acids (VLCFA) in the central nervous system. ALD has a wide spectrum of clinical manifestations, adrenomyeloneuropathy (AMN) being the most common (65%) form. AMN affects the spinal cord and leads to axonal degeneration that results in spastic paraplegia during adulthood. It is established that allogeneic hematopoietic cell transplantation (HCT) at an early stage of the human disease stabilizes or reverses cerebral demyelination. The effect of HCT to cure or prevent AMN is however unknown.The knock-out ALD mouse develops a late-onset phenotype that resembles AMN. We report that transplantation of bone marrow cells from normal mouse, known to contain progenitors of microglia, prevent the AMN phenotype of ALD mouse. The long-term follow-up to 3 patients with cerebral ALD transplanted at 8, 9 and 11 years shows that none of them have developed clinical or electrophysiological symptoms of AMN at 26 years.These data provide the first preclinical and clinical informations for the prevention of human AMN by hematopoeitic cell transplantation.

COMBINED AXONAL AND GLIAL PHENOTYPE IN THE OPTIC NERVE OF *TAG-1* MUTANT MICE

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TAG-1 is an adhesion molecule, expressed by neurons and myelinating glial cells. During development, the transient expression of TAG-1 by axons in several fascicles of the embryonic brain suggests a role for TAG-1 in axono-axonal and/or axono-glial interactions. To investigate this possible role of TAG-1, we focused our study on the embryonic optic nerve where astroglial and oligodendroglial cells develop in contact with the axons, and where TAG-1 is strongly expressed by retinal ganglion cells (RGCs) during the period of axonal growth and targeting.

In vitro, we examined the effect of TAG-1 on RGC axons and glial precursor cells. TAG-1 protein promoted RGC axon outgrowth. The trophic and adhesive responses of developing glial cells were also changed in contact with TAG-1. Recombinant TAG-1 protein strongly inhibited the adhesion of astroglial cells, but had no effect on their proliferation or survival.

We used TAG-1 deficient mice to examine the consequences of TAG1 loss on the morphology of axons and glial cells in the optic nerve. Electron-microscopy analysis of hetero and homozygous animals showed structural anomalies in axon compaction and the distribution of glial cells between the axonal bundles. In the absence of TAG-1, astroglial differentitaion in the optic nerve seems to be precipitated in time, whereas an apparently normal retino-tectal tract is established and oligodendrocyte precursor cells' colonisation of the optic nerve is normal.

Altogether our present findings indicate that TAG-1 acts on the development of both RGC axons and glial cells of the optic nerve. Further ivestigations are in progress to determine their functional importance in the context of axonal repair.

LEUKOENCEPAHLOPATHY WITH BRAIN STEM AND SPINAL CORD INVOLVEMENT AND ELEVATED WHITE MATTER LACTATE: SERIAL PROTON MR SPECTROSCOPY OF A CHILD FOR 5 YEARS

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Introduction: Leukoencephalopathy with brainstem and spinal cord involvement and elevated lactate yields distinct neuroradiologic features such as widespread, often patchy signal abnormalities on MRI of cerebral white matter (WM) and selective tract involvement to the level of the spinal cord. MR spectroscopy (MRS) reveals elevated lactate (Lac) in affected WM. Here, we report the results of a serial MRS study extending over 5.5 years of a child with this WM disorder. The study was carried out to further elucidate the underlying pathophysiological processes. Clinical History: At the age of 3 years and after normal development, a sudden gait ataxia was noted in the boy. He developed a hand ataxia shortly afterwards. For the last 4 years no clinical progression was observable and he is still ambulatory. MRI revealed the characteristic signal pattern. Methods: Localized proton MRS (3T, STEAM, TR/TE = 6000/20ms) was performed in WM and GM at 2, 2.5, 3, 3.5, and 7.5 years after disease onset. Absolute concentrations of N-acetylaspartate and N-acetylaspartylglutamate (tNAA), creatine and phosphocreatine (tCr), choline-containing compounds (Cho), inositol (Ins) and Lac were determined by LCModel and compared to a group of age-matched controls (n=7). Results: In WM tNAA was initially reduced to 3.7 mmol/l (controls: 6.9 +/- 0.6 mmol/l) but recovered to 6.5 mmol/l over the time of the study. Cho at 1.3-1.9 mmol/l (controls: 1.6 +/- 0.3 mmol/l) and tCr at 3.9-5.4 mmol/l (controls: 4.9 +/- 0.4 mmol/l) remained within normal ranges throughout. Ins showed a temporary increase to 5.9 mmol/l (controls: 3.7 +/- 0.6 mmol/l). Lac was strongly elevated to 4 mmol/l at disease onset and gradually decreased to

PREVENTION OF POST PARTUM RELAPSES WITH PROGESTIN AND ESTRADIOL IN MULTIPLE SCLEROSIS: THE POPART'MUS RANDOMIZED TRIAL

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Multiple sclerosis (MS) affects 1 in 1000 people in western countries, mainly women in their childbearing years. This autoimmune disease of the central nervous system (CNS), results in a chronic focal inflammatory response with subsequent demyelination and axonal loss. It usually begins with acute episodes of neurological dysfunction, the relapses, followed by periods of partial or complete remission. This relapsing-remitting phase is usually followed by a steady, continuous and irreversible worsening of the neurological dysfunction, which characterizes the progressive phase of the disease.

A recent prospective study reported a significant decline by two-third in the rate of relapses during the third trimester of pregnancy and a significant increase by two-third during the first three months post-partum in comparison with the relapse rate observed during the year prior to pregnancy (Confavreux et al.,*N.Engl.J. Med*, 1998, 339:285-291). These changes in the relapse rate occur at a time when impregnation of many substances, among which sexual steroids, is at its highest, before a dramatic decline, immediately following delivery.

It may be hypothesized that sexual steroids could exert beneficial effects through a modulation of the immune state with a lowering of the pro-inflammatory lymphocyte responses of the Th_1 type and an enhancement of anti-inflammatory responses of the Th_2 type. They may also play a direct role in remyelination of central nervous system lesions, (Ghoumari et al., *J.Neurochem.*, 2003, 86:848-59 ; Ghoumari et al., *Neuroscience*, 2005 , 135 :47-58 ; Ibanez et al., *Neuropathol.Appl.Neurobiol*., 2004, 30:80-89).

The POPART'MUS study is an European, multicentre, randomized, placebo-controlled and double-blind clinical trial. The expected sample size is 300 patients (150 women per group). The primary objective is to evaluate the preventive effect on post-partum MS relapses of a high dose of a 19-nor-progesterone derivative (Nomegestrol Acetate, LUTENYL®, 10 mg/day), in combination with an endometrial protective dose of transdermal estradiol. (DERMESTRIL SEPTEM 75®, one patch a week). Steroids are started within 24 hours after delivery for LUTENYL®, 2 weeks later for DERMESTRIL, and are administered continuously during the first 12 weeks post-partum. Women of the control group are given identical placebo tablets and patches with the same timing. Follow-up lasts 24 weeks with visits at 12 weeks and 24 weeks for clinical evaluation, and a continuous record of relapses. A biological and MRI follow-up will also be done in a subgroup of 60 patients. Recruitment started in France in June 2005.

Assuming the results of the trial to be positive, this new treatment could be considered in the relapsing-remitting phase of the disease in women, not only in post-partum.

(This trial is sponsored in part by the Myelin Project and ELA, the French Ministry of Health through a "Projet Hospitalier de Recherche Clinique" (PHRC 2005) and the French Multiple Sclerosis Society (ARSEP).

CHOLESTEROL DEPLETION INCREASES MONO-UNSATURATED VERY LONG CHAIN FATTY ACIDS IN X-ALD

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Introduction
X-linked adrenoleukodystrophy is a metabolic disorder characterized by the accumulation of very long-chain fatty acids (VLCFA; > C22) and reduced beta-oxidation of VLCFA in peroxisomes. It is caused by mutations in the *ABCD1* gene, which codes for ALD protein (ALDP). Previous studies have reported that intracellular cholesterol depletion reduces C26:0 levels, possibly by SREBP-mediated increased expression of the ALDP-related protein (ALDRP) and increased peroxisomal beta-oxidation.
Methods
Skin fibroblasts from patients with X-ALD were cultured on HAMF10 culture medium with either 10% fetal calf serum (FCS), 10% delipidated FCS (containing no cholesterol) or 10% delipidated FCS supplemented with cholesterol and 25-OH-cholesterol. Stable isotope labeled fatty acids were added to measure activity of specific metabolic pathways. The effect of cholesterol depletion on the expression of genes of interest was analyzed by LightCycler.
Results
Depletion of cholesterol resulted in a slight reduction in endogenous C26:0, but we also observed a strong increase in endogenous C26:1 levels as a result of increased *de novo* synthesis of C26:1. In line with this, the expression of stearoyl-CoA-desaturase type 1 is increased on delipidated FCS and its activity (conversion of C18:0 to C18:1) increased. The β-oxidation of C24:0 is strongly reduced when cells are cholesterol depleted.
Conclusions
We conclude that cholesterol depletion results in a slight reduction in C26:0 levels. This effect, however, is accompanied by a shift in *de novo* fatty acid synthesis from saturated towards mono-unsaturated fatty acids.

A CRITICAL LOCUS RELATED TO A NEW LEUCODYSTROPHY WITH PROGRESSIVE ATAXIA, DEAFNESS AND CARDIOMYOPATHY IN A CONSANGUINOUS FAMILY

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We reported on three patients, two girls and a boy, of a large consanguineous family from southern Italy, affected by a distinctive leukodystrophy with clinical phenotype characterised by slowly progressive ataxia and spasticity with an onset between 2 and 3 years of age. After 5-6 years of disease evolution, patients experienced sensorineural deafness, resulting in complete hearing loss in the time-span of 2 years. Subsequently, they developed a restrictive cardiomyopathy after age 13 years responsible for a rapid death in 2 patients. Brain MRI demonstrated, in all cases, a diffuse abnormal signal of the withe matter on T2. An increase in hepatic enzymes was observed for the three patients but an extensive neurometabolic assessment failed to detect any abnormalities. We found a region linked to the disease on chromosome 1 (1p34.3-p33). This large region of 9 cM, contains 176 genes (108 known and 68 EST). We analyzed the coding sequence and flanking intronic regions of 41 genes in one or two of the propositi. No mutation was found. We identified several heterozygote alleles in genes sequenced SNPs allowing to reduce the region to 3.11 cM. Sixty-nine genes have been assigned to this 3.11 cM interval, 25 genes as highly candidate and 12 are already sequenced and negative. In case of gene identification, functional analysis will be performed and this new gene will be tested in the large group of undetermined leukodystrophies according to the MRI features.

PROTEOMIC/PEPTIDOMIC-BASED BIOMARKER STUDIES FOR CACH/VWM LEUKODYSTROPHY

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Childhood ataxia with central hypomyelination/vanishing white matter leukodystrophy (CACH/VWM) is characterized by a CSF-like white matter on cerebral MRI, by a large clinical spectrum from rapidly fatal infantile forms to asymptomatic adult forms and by sensitivity to stress events. This disorder has been linked to mutations in the five genes encoding the subunits of the ubiquitous eukaryotic initiation factor 2B (eIF2B) involved in protein synthesis and its regulations upon stress. The intrinsic activity of the eIF2B complex is known to be specifically decreased in lymphoblasts from affected patients. In order to understand the patho-physiology of CACH/VWM and to analyze the functional consequences of eIF2B mutations, we used differential display strategies to investigate the peptidome and proteome of mutated lymphoblasts from six affected patients in comparison to control samples. Following two-dimensional gel electrophoresis and mass fingerprints, no differences was found in the proteome of mutated lymphoblasts in comparison with age matched controls. In parallel, liquid chromatography-based peptidomics studies revealed one potential candidate up?regulated in 5/6 mutated-lymphoblasts peptidomes (Fogli et al., 2006). However, analysis of this potential biomarker in a larger number of control samples did not confirm its disease specificity. We subsequently optimized, on the P4M plateform (see the poster presented by Perret et al.), conditions for peptidomic analysis of human body fluids (plasma, serum, urines and CSF) and tissues (brain). Differential peptidomic analyses on 14 plasma samples originating from eIF2B-mutated patients in comparison to controls and using the methods developed for sample fractionation are currently under investigation.

VALPROIC ACID STIMULATES *ABCD2* GENE EXPRESSION: A NOVEL POTENTIAL THERAPY FOR X-ADRENOLEUKODYSTROPHY

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Our ultimate goal is to develop new therapies for X-linked adrenoleukodystrophy (X-ALD), the most frequent inherited monogenic demyelinating disease (minimal incidence 1:17,000). X-ALD leads to death in boys due to cerebral demyelination (cerebral childhoold ALD, CCALD) or to motor disability in adults due to spinal cord and peripheral nerve degeneration (adrenomyeloneuropathy or AMN). The gene mutated in the disease (ABCD1) is a peroxisomal ATP-binding transporter of very long-chain-fatty acids, whose accumulation is the hallmark of the disease. We have generated and characterized mouse models for X-ALD by inactivation of ABCD1 and of a close homolog, the ABCD2 peroxisomal transporter. Recently, we have shown that stable overexpression of ABCD2 is able to prevent the late-onset neurodegenerative phenotype presented by ABCD1 knock-out mice. This constitutes an *in vivo* evidence of the overlapping functions of both transporters in the mouse. Because ABCD2 is a target of histone deacethylator (HDAC) inhibitors such as 4-phenylbutyrate, we investigated the effect of valproic acid (VPA), an HDAC inhibitor successfully used for the long-term treatment of epilepsy. Indeed, VPA stimulates ABCD2 expression *in vitro* and *ex vivo* in mouse and human. When given to X-ALD patients, a 2 to 4 fold upregulation of ABCD2 in peripheral mononuclear cells in 50% of the patients is reached. Thus, our findings open encouraging perspectives for the therapy of this devastating disease.

CDK2 LOSS PREFERENTIALLY AFFECTS ADULT SVZ PROGENITOR CELL DEVELOPMENT - COMPARISON WITH EARLY POSTNATAL PROPERTIES

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The G1-S phase transition of the cell cycle is regulated by cyclin-dependent kinases and by their inhibitors. Cdk2 plays a crucial role in the control of oligodendrocyte progenitor cell (OPC) proliferation. Here, we have shown that, in adult (P90) the subventricular zone (SVZ) and ependymal layer of lateral ventricle - genetic deletion of Cdk2 affected cell proliferation, resulting in reduction in the number and the proliferation rate of NG2-expressing progenitor cells. Conversely, at early postnatal stages (P8-10), no differences between WT and Cdk2-/- mice were observed either in the total number of SVZ proliferating cells, or in the number of NG2+BrdU+ cells. Lineage potential of NG2+ progenitors showed that the lack of Cdk2 promoted their differentiation in adult mice, as demonstrated by a reduction in NG2+nestin+ cells and an increase in the number of oligodendrocyte-committed progenitors (Nkx2.2+) and neuroblasts (Dcx+). In vitro studies using SVZ cell cultures demonstr ated that neurosphere formation in adult Cdk2-/- cells was significantly decreased as compared to WT, whereas cell differentiation into oligodendrocytes, astrocytes and neurons was significantly increased. Western Blot analysis in SVZ tissue demonstrated a significant up-regulation of Cdk4, Cdk6, Cyclin-D, Rb, E2F-1 and p21 protein expression in early postnatal Cdk2-/- tissue only. A possible compensatory mechanism involving Cdk4 in SVZ cell proliferation is currently being studied by knockdown of Cdk4 in P8 Cdk2-/- neurospheres. Our data indicate that Cdk4 might compensate the absence of Cdk2 in SVZ progenitors during early postnatal development, whereas this compensation does not occur in adult SVZ progenitor cells. Supported by a grant form the European Association Against Leukodystrophies(ELA)

CELLULAR AND ANIMAL MODELS FOR eIF2B-RELATED LEUKODYSTROPHY

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Childhood ataxia with central nervous system hypomyelination (CACH), also known as vanishing white matter (VWM), an autosomal recessive disease is caused by mutations in each of the five subunits of translation initiation factor 2B (eIF2B). ATF4 mRNA translation is hyper-induced in primary fibroblasts derived from CACH/VWM patients upon ER-stress (Kantor et al., 2005). Myelin loss in the CNS of CACH/VWM patients is attributed mainly to loss of oligodendrocytes function. Therefore, we set to generate an oligodendrocytic cellular model to study the disease, using siRNA to stably knock-down the endogenous eIF2B epsilon gene (eIF2B5) in rat oligodendrocytes DDR1 cell line. eIF2B5 knock-down had no significant effect on global protein synthesis rate, or on ATF4 induction levels in response to ER-stress, but showed a decrease in cells viability under ER-stress. Human WT or mutated (R195H) eIF2B5 over-expression in the knocked-down clones resulted in increased ATF4 and GADD34 induction even in the absence of stress, indicating that high eIF2B5 level induces stress by itself.

To generate a more authentic model for CACH/VWM, we decided to create an animal model which will serve as a source for all types of brain cells. For the knock-in (KI) model, the R132H mutation (corresponding to human R136H mutation) in eIF2B5 was introduced into the genome of mouse ES cells. A knock-out (KO) model was also generated, by insertion of the Neo cassette into exon6 of eIF2B5 gene. Heterozygous mice harboring the KI or the KO allele were generated. Homozygous KI/KI or compound heterozygous KI/KO mice will serve as animal models for further experiments.

VIRCHOW-ROBIN-SPACES ON MAGNETIC RESONANCE IMAGES OF CHIDLREN WITH ADRENOLEUKODYSTROPHY

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Perivascular spaces, also known as Virchow-Robin spaces (VRS), have been described as structures with immunological and neuropathological significance. In Adrenoleukodystrophy (ALD) the disease process has been linked to the perivascular location with immunoreactivities involving perivascular cells. We therefore hypothesised that VRS are of importance in the disease process in ALD and can be visualised by MRI.

MRI and clinical data from 35 patients with the cerebral form of X-linked ALD, 29 with the asymptomatic form (including those with adrenal insufficiency), and 36 control patients were studied retrospectively to elucidate the clinical significance of VRS on MRI for boys with ALD and to question whether VRS could be of prognostic or diagnostic relevance in the evaluation of the onset or severity of the clinical course.

VRS could be visualised by MRI in 87% of patients with asymptomatic ALD, in 80% of control patients, and in 47% of patients with cerebral ALD. None of them were found to be dilated. The number of visible VRS correlated negatively with the degree of demyelination both in patients with the cerebral and the asymptomatic form. Furthermore, in the group of patients with cerebral ALD the number of visible VRS correlated positively with a less severe disease progression.

It is therefore possible to speculate that the appearance or a higher number of visible VRS in ALD is associated with an earlier stage of the disease, or even a more benign clinical course. VRS on MRI of patients with ALD seem to reflect the perivascular inflammatory process of this disease.

WEB ACCESS PLATFORM FOR RESEARCH ON LEUKODYSTROPHIES

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The large clinical and genetic heterogeneity of leukodystrophies needs medical and scientific data collections to optimise diagnosis and follow-up of affected patients and their families. The multiple origins of the data require a centralised database. We developed a „leukodystrophies‰ database on the web access solution created by HC Forum (www.hcforum.fr). This highly secured platform allows individual and family data acquisitions according to the family tree including informations on clinical signs, neuroimaging, electrophysiology, neuropathology, biology and samples collected∑ In addition, we created a specific workflow to allow collaborative studies within the multidisciplinary LeukoFrance network. The distributed expertises generated through this network, is used for diagnosis help, patients follow-up and research projects. Research programs on the epidemiology of leukodystrophies and the natural history of PLP mutated patients has been initiated and can be applied to other purposes like therapeutic trials, gene identification. Access to and sharing of informations are individualised according to users and their role in order to protect the medical data confidentiality and work within the network. We will present the tools we have developed on the HC Forum platform dedicated to research on leukodystrophies. We will chiefly focus on the generic aspect of our approach and on the modular architecture of the database.

DIFFERENT SUBTYPES OF NG2 POSITIVE GLIA IN THE WHITE MATTER OF RAT CEREBELLAR SLICES.

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For normal function of the nervous system, oligodendrocytes are needed to speed the propagation of the action potential. In diseases like cerebral palsy, spinal cord injury and multiple sclerosis, oligodendrocytes get damaged and the action potential is slowed or abolished. Replacement of damaged oligodendrocytes, either by endogenous or perhaps transplanted cells, is essential to restore normal function.

In the adult brain more than 5% of the cells are glia that express the proteoglycan NG2 (Dawson et al., 2003). The function of these cells is poorly understood. They are generally thought to be oligodendrocyte precursor cells, which might replace myelinating oligodendrocytes that become damaged. However, they have recently been suggested to be either multipotent stem cells able to generate both GABAergic neurons and oligodendrocytes (Aguirre et al., 2004), or a new type of glia called synantocytes which contact the nodes of Ranvier (Butt et al., 1999). Because of this controversy over their function, we have examined the origins of these cells with immunohistochemistry and studied their electrical properties by whole-cell clamping. Dye filling from the whole-cell pipette allowed us to study the cells' morphology and recover the recorded cell for antibody labelling (Káradóttir et al, 2005).

In the white matter of cerebellar slices at postnatal day 7, most NG2 glia are part of the oligodendrocyte lineage: 93% of 106 cells which labelled with antibody to NG2 were also labelled by antibody against the oligodendrocyte transcription factor Olig2. However, these NG2/Olig2 positive cells could be divided into two subtypes with distinct electrophysiological properties. One subtype showed a TTX-sensitive sodium current, followed by a slowly developing outward rectifying current presumably mediated by voltage-gated potassium channels, in response to depolarizing voltage steps. The other subtype did not show these voltage-gated currents. Interestingly, although both subtypes responded to superfused GABA and glutamate, only the subtype expressing the voltage-gated channels also received action potential driven synaptic input, both GABA-ergic and glutamatergic, which was TTX-sensitive.

These data suggest that two subtypes of oligodendrocyte precursor exist, with distinct electrophysiological characteristics, contrary to current assumptions. Only the subtype expressing voltage-gated channels senses its neuronal environment by receiving synaptic input from passing axons. Conceivably one of these subtypes may be destined to myelinate axons immediately, and the other may become the "adult precursors" which can differentiate into myelinating oligodendrocytes after pathological conditions.

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DYSMYELINATION AND RECOVERY ASSESSMENT IN VIVO BY DIFFUSION TENSOR MAGNETIC RESONANCE IMAGING IN TRANSGENIC MICE

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Diffusion tensor magnetic resonance imaging (DT-MRI) was applied for in vivo quantification of myelin loss and recovery. A transgenic mouse line (Oligo-TTK) expressing a truncated form of the herpes simplex virus 1 thymidine kinase gene (hsv1-tk) in oligodendrocytes was studied along with two induced phenotypes of myelin pathology. Changes in the anisotropy of the white matter were assessed by calculating and mapping the radial (D%4) and axial (D//) water diffusion to axonal tracts and fractional anisotropy (FA). A significant increase in D%4 attributed to the lack of myelin was observed in all selected brain white matter tracts in dysmyelinated mice. Lower D// values were consistent with the histological observation of axonal modifications, including reduced axonal caliber and neurofilaments overexpression. The myelination and axonal changes play a significant role in the degree of diffusion anisotropy, because FA was significantly decreased in dysmyelinated brain. Importantly, myelin reparation during brain postnatal development induced a decrease in the magnitude of D%4 and an increase in FA compared with the same brain before recovery. The progressive increase in D// values was attributed to the gain in normal axonal morphology. The myelin reparation was confirmed by the detection of enlarged oligodendrocyte population, newly formed myelin sheaths, and a gradual increase in axonal caliber.

NONINVASIVE IN VIVO IMAGING OF MYELIN DEFECTS AND RECOVERY IN A MOUSE MODEL OF PMD

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Pelizaeus-Merzbacher Disease (PMD) is a disorder linked to mutations of PLP/DM20 gene on X chromosome producing an irreversible hypomyelination in males. The jimpy mouse, a model of human PMD, was used for in vivo brain diffusion tensor magnetic resonance imaging (DT-MRI). Affected males and heterozygous females were used to analyze noninvasively, the differential effect of the PLP mutation on the white matter. Quantification of the dysmyelination in jimpy males was also compared to myelin loss induced by oligodendrocytes cell death in transgenic mice (Oligo-TTK). Changes in DT-MRI parameters were supported by histological examination. The radial ($D_{\perp}$) and axial ($D_{//}$) water diffusion to axonal tracts; fractional anisotropy (FA) and mean diffusivity (<D>) were calculated and mapped. A significant increase of $D_{\perp}$, caused by the lack of myelin was observed in the white matter of jimpy and Oligo-TTK males. Interestingly, the great elevation of $D_{\perp}$ was accompanied by a slight increase of $D_{//}$ values in jimpy, in contrast to a significant decrease of $D_{//}$ values observed in transgenic mice. The severe astrocytic hypertrophy and the overexpression of AQP4 protein noticed in jimpy males may explain the differences obtained in the two models. DT-MRI of the jimpy brains in heterozygous females allowed monitoring myelin recovery.

HOMO-AND HETERODIMERIZATION OF THE HUMAN ADRENOLEUKODYSTROPHY PROTEIN (ALDP; ABCD1) IN THE PEROXISOME MEMBRANE

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X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disorder biochemically characterized by elevated levels of very long chain fatty acids due to impaired peroxisomal ß-oxidation. The primary cause is mutations in the adrenoleukodystrophy gene. The gene product, the adrenoleukodystrophy protein (ALDP), is one of four known half ATP-binding cassette (ABC) transporters in the peroxisomal membrane. Besides ALDP PMP70, ALDR and P70R have been identified. The interactions of ALDP within the peroxisomal membrane as well as the role and function of ABC-transporters in disease pathogenesis are poorly understood. In general, ABC half transporters have to dimerize to gain functionality. For the dimerization of ALDP so far conflicting observations have been made. By means of yeast two-hybrid assays and co-immunoprecipitation analysis it was shown that ALDP forms homo- as well as heterodimers with PMP70 and ALDR while other studies revealed only homodimer formation. To circumven t the problems of artificial interactions due to biochemical sample preparations in vitro, we investigated the protein-protein interactions of ALDP in its physiological environment applying FRET microscopy in intact living cells. We could show in vivo that ALDP forms homodimers and heterodimers with PMP70. Moreover by using C-terminal deletion constructs of ALDP we could show that the last 87 C-terminal amino acids are important for these interactions, and that the N-terminal transmembrane region of ALDP has a stabilizing effect on homodimerization. The statistical relevance of the FRET data was determined in two different ways using probability distribution shift analysis and Kolmogorov-Smirnov statistics. In conclusion, the loss of ALDP homo- or heterodimerization due to mutated ALDP is surely relevant for understanding the disease mechanisms of X-ALD.

A ROLE FOR VEGFR-3 IN NEURAL CELL DEVELOPMENT

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The central nervous system (CNS) development requires continuous interactions with the blood vascular network for nutrients and oxygen supply. Nerves growth is closely related to vessels branching. Recent studies have proven that vascular growth factors can target neural cells. In fact, VEGF-A (Vascular Endotelial Growth Factor A) has been implicated in neural development and neurodegenerative diseases such as ALS. We have shown that another member of this family, the lymphangiogenic factor VEGF-C can act on subpopulations of neural precursors cells during embryonic development of both Xenopus and mice (Le Bras et al., 2006). VEGF-C knocked-out Xenopus and mice have defective CNS development due in part to a decreased neural progenitors proliferation. In vitro, VEGF-C stimulates proliferation and migration of neural precursor cells. To better understand the role of VEGFR-3 in the embryonic CNS, we propose to specifically delete VEGFR-3 expression by RNA interference in discrete territories of the CNS. The chicken is the most convenient model for this study. VEGF-C and its receptors VEGFR-2; -3 and Npn2 expression has been verified by ISH. The strongest signal is located in the thalamus from HH 25 (E4.5) and is still present at HH38 (E12). We are now testing siRNA to silence vegfr-3 expression. SiRNAs will be injected and electroporated in the thalamus at HH 22/23, in ovo. The embryo will be allowed to develop for 2 more days. The phenotype will be examined at HH 29. This approach will enlighten the setting of the VEGFR-3 expressing neural progenitors during the CNS development.

FUNCTIONAL CONSEQUENCES OF EUKARYOTIC INITIATION FACTOR 2B MUTATIONS IN CACH/VWM DISEASE.

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The Childhood Ataxia with diffuse Central nervous system Hypomyelination (CACH)/Vanishing White Matter (VWM) syndrome is an autosomal recessive leukodystrophy characterized by a progressive spastic ataxia and a CSF like aspect of the white matter on MRI Severity is correlated with age at disease onset and with episodes of rapid deterioration following febrile infection or head trauma. Mutations in the five subunits of the eIF2B translation initiation factor (α to ε) are the most frequent cause of the disease. eIF2B is involved in the first step of protein synthesis by activating the translation initiation factor eIF2 thanks to its guanine nucleotide exchange activity (GEF activity). By regeneration of active eIF2 , eIF2B is a key factor in the cellular stress response. We demonstrated that GEF activity of eIF2B is decreased in CACH/VWM patients lymphoblastoid cell lines in correlation with disease severity. In order to test the diagnostic interest of this GEF activity, we measured it in lymphocytes and fibroblasts. Moreover, functional consequences of eIF2B mutations on the reticulum endoplasmic stress response have been studied in 12 mutated lymphoblasts in comparison between 6 controls treated with thapsigargin. The transcripts variation of four stress-dependent genes *ATF4*, *CHOP*, *ASNS* and *BiP* determined by quantitative RT-PCR was not significantly different between mutated and non-mutated cells suggesting that an abnormal transcriptional activation of these genes in the stress conditions tested was not present in mutated cells. Then stress conditions and cellular models have been optimized in order to start a transcriptomic study on 20 couples mutated patients versus controls.

HEIGHTENED STRESS RESPONSE IN FIBROBLAST EXPRESSING MUTANT EIF2B GENES FROM CACH/VWM PATIENTS

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Childhood ataxia with central nervous system hypomyelination (CACH), also called vanishing white matter (VWM) leukoencephalopathy, is a fatal genetic disease caused by mutations in eukaryotic initiation factor 2B (eIF2B) genes. The five subunits eIF2B factor is critical for translation initiation under normal conditions and regulates protein synthesis in response to cellular stresses. Primary fibroblasts from CACH/VWM patients and normal individuals were used to measure basal eIF2B activ ity as well as global protein synthesis and ATF4 induction in response to stress in the endoplasmic reticulum. We show that although the cells expressing mutant eIF2B genes respond normally to stress conditions by reduced global translation rates, they exhibit significantly greater increase in ATF4 induction compared to normal controls despite equal levels of stress and activity of the upstream eIF2Éø kinase. This heightened stress response observed in primary fibroblasts that suffer from minor loss of basal eIF2B activity may be employed as an initial screening tool for CACH/VWM leukodystrophy.

ELOVL1 is the very long-chain fatty acid specific elongase

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X-linked adrenoleukodystrophy (X-ALD) is a progressive neurodegenerative disorder that is characterized by the accumulation of very long-chain fatty acids (VLCFA) and reduced degradation of VLCFA in peroxisomes. Recently, we demonstrated that chain elongation of VLCFAs to even longer fatty acids is enhanced in fibroblast cell lines from X-ALD patients. This points to an imbalance in the fatty acid homeostasis in X-ALD and has generated new perspectives with respect to therapeutic intervention for X-ALD based on the inhibition of the synthesis of VLCFA.
Over 90% of fatty acids in humans have a chain length of 16 to 18 carbon atoms and are derived from *de novo* synthesis. Subsequent elongation to VLCFAs is carried out by endoplasmic reticulum associated, membrane bound, fatty acid chain-elongation enzymes (elongases). In the last years, seven elongases (ELOVL) have been identified in human. Elongases are present in a wide variety of species including: plants, nematodes, yeast, fish and mammals. While it is known that ELOVL enzymes are involved in the synthesis of saturated, mono-unsaturated and poly-unsaturated fatty acids, their individual substrate specificities, however, have not yet been identified. To find the elongase involved in the synthesis of VLCFA, we cloned all seven human ELOVLs and used several strategies, including heterologous expression in yeast and Chinese hamster ovary (CHO) cells and expression in human fibroblasts. We identified ELOVL1 as the only elongase that has substrate specificity towards VLCFAs.

SILENCING THE JIMPY PROTEOLIPID PROTEIN GENE PARTIALLY CORRECTS OLIGODENDROCYTE PHENOTYPE.

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Jimpy (jp) is a point mutation in the proteolipid protein (PLP) gene that causes premature oligodendrocyte (OL) death and CNS dysmyelination in affected mice. It accurately models certain Pelizaeus-Merzbacher disease (PMD) mutations. Along with their inability to produce myelin and ensheathe axons, jp OLs exhibit abnormalities including elevated $[Ca^{2+}]_i$, protracted cell cycling, and altered membrane potential. The molecular events responsible for jp abnormalities are not clearly understood. Jp OLs cannot produce normal PLP and may, additionally, make an altered form of the protein (jpPLP). Since expression of normal PLP by jp OLs failed to normalize their phenotype, we tested whether silencing jpPLP might be a successful strategy. Initial studies used cell lines immortalized from normal (158N) and jp (158JP) OLs. Stable cell lines were derived after transfection with DNA based siRNA vectors for jpPLP. Resting membrane potential, $[Ca^{+2}]_i$, expression of certain cAMP/Ca^{2+} related genes, and dbcAMP-induced responses were normalized in silenced 158JP cells. In addition, CNPase expression was upregulated. These effects, as well as cell survival, are currently being evaluated in primary jp OLs. Our evidence suggests that silencing may be a useful approach in the management of dominant-negative PLP mutations causing PMD. Support: ELA (MSG&PEK).

PERTURBED INTERACTIONS OF PLP/DM20 WITH CHOLESTEROL AND LIPID RAFTS IN OLIGODENDROGLIA: IMPLICATIONS FOR PELIZAEUS-MERZBACHER-DISEASE AND SPASTIC-PARAPLEGIA.

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Missense mutations in the human *PLP1* gene lead to different forms of dysmyelinating Pelizaeus-Merzbacher-Disease (PMD) or the milder Spastic-Paraplegia-Type-2 (SPG-2). The molecular pathology has been generally attributed to ER-retention of misfolded proteolipid protein (PLP and its splice isoform DM20) and induction of the unfolded protein response (UPR). As opposed to previous studies of heterologous expression systems, we have analysed PLP/DM20 trafficking in oligodendroglial cells, thereby revealing differences between PMD and SPG-2-associated PLP/DM20 isoforms. PLP^{A242V} and $DM20^{A242V}$, associated with severe PMD-like phenotype *in vivo* were not only retained in the ER, but also induced the retraction of oligodendroglial processes. In contrast, glial cells expressing SPG-2-associated PLP^{I186T} or $DM20^{I186T}$ developed processes, and mutant PLP/DM20 reached a late endosomal/lysosomal compartment. Unexpectedly, PLP/DM20 with either substitution exhibited impaired cholesterol binding, and the association with lipid raft microdomains was strongly reduced. Turnover analysis demonstrated that mutant PLP was rapidly degraded in oligodendroglial cells, with half-lifes for PLP > PLP^{I186T} > PLP^{A242V}. Protein degradation was specifically sensitive to proteasome inhibition, although $PLP/DM20^{I186T}$ degradation was also affected by inhibition of lysosomal enzymes. We conclude that in addition to ER retention and UPR induction impaired cholesterol binding and lipid raft association are characteristic cellular defects of *PLP1*-missense mutations. Mutant protein is rapidly cleared and does not accumulate in oligodendroglial cells. While UPR induced cell death governs the PMD phenotype of the *msd* mutation, we propose that impaired cholesterol and lipid raft interaction of the *rsh*-protein is etiologic to the dysmyelination observed in SPG-2. Supported by ELA.

IN VITRO PROTON MAGNETIC RESONANCE SPECTROSCOPY OF JIMPY MICE (*PLP1*^{JP}/Y) BRAIN: EARLY METABOLIC ALTERATIONS IN A PELIZAEUS-MERZBACHER DISEASE MODEL DURING POSTNATAL MATURATION.

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ABSTRACT

The Jimpy mouse (*Plp1*^{JP}/Y) is a *plp1* dysmyelinating mutant mouse model of Pelizaeus-Merzbacher disease (PMD). The apoptotic death of mature-myelinating oligodendrocytes (Ols) that produce *PLP1* proteins is responsible for the severe hypomyelination with early death (<1 months) observed. *In vitro* proton magnetic resonance spectroscopy (MRS) was used to look for markers of this pathological process. For this purpose, the brain metabolic profiles of *Plp1*^{JP}/Y mice and normal +/Y mice from the same littermates were compared.

Wild type male (+/Y) *versus* jimpy male (*Plp1*^{JP}/Y) were sacrified at different postnatal (P) ages: P1, P5, P10, P15 and P22. The brain were quickly removed (< 1 min) frozen in liquid nitrogen and stored at – 80° C for perchloric acid extraction. The ^{1}H NMR spectra were recorded on a Bruker Avance 400 (9.4 T). Absolute metabolite quantification of 7 metabolites: *N*-acetylaspartate (NAA), total choline (tCho), total creatine (tCr), glutamate (Glu), taurine (Tau), Gamma-aminobutyric acid (GABA) and *N*-acetylaspartylglutamate (NAAG) have been performed.

Variations of these different metabolites during brain development were in accordance with those previously reported in mice using in vivo MRS studies (*see poster Larvaron P et al*). The evolutions of metabolite concentrations as a function of age were similar for the two genotypes. Significant differences ($p<0,01$) between the two genotypes were observed for 3 metabolite (Tau, tCr, tCho) concentrations mostly between D1 and D5. Tau ($p<0.01$) and tCho ($p<0.05$) were the two metabolites which discriminate the genotypes. A comparison of metabolite concentration between the two genotypes at each age pointed out significant differences at P1 for Tau ($p<0.01$) and tCr, NAA, NAAG ($p<0.05$). At P5, tCr were still discriminant ($p<0.05$) as well as tCho. The *Plp1*^{JP}/Y mice showed the lowest concentrations. No significant differences between genotypes were detected at P10, P15 and P22 suggesting that the abnormalities found could be early markers of the abnormal Ols maturation. These studies will help to better understand the conflicting SRM analysis reported in PMD patients

PLP-KO MOUSE MODEL OF OLIGODENDROCYTE-DEPENDENT AXONOPATHY : BRAIN CHARACTERIZATION BY MRI AND MRS

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PLP-Ko mice represent an unique model of oligodendrocyte-dependent axonopathy with a long survival and normal fertility, relevant for human dysmyelinating diseases to test molecules of therapeutic interests. In order to better characterize this axonopathy with non invasive *in vivo* techniques, we analysed 6 PLP KO mice (Plp-/Y) in comparaison with 6 wild-type mice (Plp+/Y) at 14 months by brain magnetic resonance imaging (MRI) and spectroscopy (MRS). Rota-rod and horizontal bar tests demonstrated the severe motor deficit observed in PLP -/Y mice at this stage of the disease. The water diffusion characteristics have been studied *in vivo* by DTI. MRI pointed out an atrophy for the Plp1-/Y whole brain. In the sagittal MR images, a significant decrease (about 20%) in cortex area was observed in Plp1-/Y brain in comparison with control brain. Moreover an atrophy of fiber tracts in anterior commissure (AC) and genu of the corpus callosum (GCC) were observed in parrallel with a microstructure alteration measured by DTI: fractional anisotropy (FA) distinguished genotypes for AC, GCC ans thalamus structures. Plp1-/Y neuropathological analysis in the same structures showed oligodendrocytes hyperplasia, axon swelling and abnormal myelin compaction.Variations in metabolic profiles measured by MRS performed in a single voxel (2.2 µl) in medulla could be related with changes in brain cell composition.

IN VIVO ANALYSIS OF POST-NATAL DEVELOPMENT OF NORMAL MOUSE BRAIN BY DIFFUSION TENSOR IMAGING AND PROTON MAGNETIC RESONANCE SPECTROSCOPY

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NMR provides a non-invasive tool for the phenotypic characterisation of mouse models. The aim of the present study was to apply reliable in vivo MRS techniques for non-invasive investigations of brain development in normal and transgenic mice, by monitoring metabolite concentrations in different brain regions. The conditions of anaesthesia, immobilisation and respiratory monitoring were optimized to carry out in vivo MRS studies in young mice. All the experiments were performed in normal mice, at 9.4 T, applying a point-resolved spectroscopy (PRESS) sequence (TR ¼ 2000 ms; TE ¼ 130 ms). We obtained reproducible in vivo 1H NMR spectra of wild-type mouse brains as early as post-natal day 5, which allowed us to follow brain maturation variations from post-natal days 5 to 21. The survival rate of animals was between 66 and 90% at post-natal days 5 and 21, respectively. Developmental changes of metabolite concentrations were measured in three brain regions: the thalamus, a region rich in cell bodies, the olfactory bulb, rich in fibre tracts actively myelinated during brain maturation, and the cerebellum. The voxel size varied from 2 to 8 μl according to the size of the brain structure analysed. The absolute concentrations of the total creatine, taurine, total choline, Nacetylaspartate and of the glutamate/glutamine pool were determined from 1H NMR spectra obtained in the different brain regions at post-natal day 5, 10, 15 and 21. Variations observed during brain development were in accordance with those previously reported in mice using ex vivo MRS studies, and also in rats and humans in vivo. Possibilities of longitudinal MRS analysis in maturing mice brains provide new perspectives to characterise better the tremendous number of transgenic mutant mice generated with the aim of decrypting the complexity of brain development and neurodegenerative diseases but also to follow the impact of environmental and therapeutic factors.

The water diffusion characteristics of wild-type mouse brains have been studied in vivo by DTI to follow developmental changes. Here, axial (λ//) and radial (λ⊥) diffusivities and fractional anisotropy were measured from the fifth day of life (P5) and at three others post-natal ages (P12, P19 and P54). Magnetic resonance images have been collected from a single sagittal slice in the middle of the two hemispheres; ROI have been chosen in 9 different structures of both grey and white matter. Since P5, the fractional anisotropy (FA) has enabled distinguishing structures of white matter and grey matter even if myelination has yet to occur. Between P5 and P54, a significant decrease of FA was observed in the genu of the corpus callosum because of a significant decrease of λ⊥ whereas λ// remained stable. Many others significant variations of λ// and λ⊥ were measured in different structures and were substantially correlated with axon and myelin maturation which are responsible of the main evolutions of the brain during its post-natal development. These quantitative data show that the in vivo characterisation of anatomy and microstructure of the normal mouse brain during development is possible. This normative data will greatly improve the characterisation of abnormal development of the transgenic mouse brain.

INTRA-CEREBRAL HEMORRHAGE IN NEONATAL MICE WITH KNOCK-OUT OF PLASMINOGEN ACTIVATOR INHIBITOR-1 (PAI-1)

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Intra-cerebral hemorrhage is a frequent cause for white matter (WM) lesions in preterm neonates. Very few are known on the relationship between coagulation-fibrinolysis equilibrium unbalance and bleeding in these infants. Experimental hemorrhage was produced in genetically engineered mice carrying inactivation of the plasminogen activator inhibitor-1 (PAI-1). A mechanical lesion was produced in 5 day-old (P5) or P10 mice by intra-cortical (i.c.) injection of 2 µl sterile phosphate buffer. Macroscopic semi-quantitative grading of hemorrhage and routine histology were performed in brains at different times post-injection. i.c. PBS in P5 pups produced small sized lesions within 3 days in control and hemorrhage in PAI-1-/- mice. In the following days 4 and 5 post-injection, control mice recover while PAI-1-/- mice re-experienced bleeding. Adult PAI-1-/- mice treated at P5 exhibited WM atrophy significantly larger than control mice

METABOTROPIC GLUTAMATE RECEPTORS ARE DEVELOPMENTALLY REGULATED IN THE OLIGODENDROGLIAL LINEAGE AND ARE INVOLVED IN CELL SURVIVAL

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Oligodendrocytes (OLs) myelinate axons and are the principal cells targeted in white matter injury in the preterm brain. The cellular and molecular mechanisms involved in white matter development and immature OL injury are incompletely understood. Metabotropic glutamate receptors (mGluRs) modulate neuronal development and survival, and have recently been identified in oligodendrocyte progenitor cells (OPCs) (1, 2). Using the CG-4 OPC line and O4 marker-immunoselected primary OLs, we established the differentiation stage-specific expression profile of mGluR3 and mGluR5 mRNAs and proteins in the oligodendroglial lineage and type-2-astrocytes (ASTs). Quantitative analysis indicated no changes in mGluR3, but a significant downregulation of mGluR5a mRNA and protein expression when OPCs differentiated into OLs or ASTs. The downregulation of mGluR5a had functional consequences, with significantly fewer OLs and ASTs (compared to OPCs) responding to the group I mGluR agonist DHPG with intracellular Ca2+ oscillations. Neither stimulation nor inhibition of mGluR3 or mGluR5 altered OPC migration, suggesting that mGluRs are not involved in the regulation of OPC motility. Activation of mGluR5 during staurosporine-induced apoptosis resulted in total survival in OPCs and a significant reduction in apoptosis in OLs, suggesting that the downregulation of mGluR5 in premyelinating OLs may contribute to their increased vulnerability. Targeting mGluR5 may be a potential therapeutic strategy to promote oligodendroglial survival. 1. Luyt K et al. Functional metabotropic glutamate receptors are expressed in oligodendrocyte progenitor cells. J Neurochem. 2003;84(6):1452-64. 2. Luyt K et al. Metabotropic glutamate receptors are expressed in adult human glial progenitor cells. Biochem Biophys Res Commun. 2004;319(1):120-9.

INFECTION OF HUMAN NEUROSPHERES BY LENTIVRUS ENCODING FOR OLIG TRANSCRIPTION FACTORS

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Olig genes are bHLH transcription factors involved in oligodendrocyte differentiation during central nervous system (CNS) development. Olig2 is necessary and sufficient for oligodendrocyte specification, while Olig1 is required for myelination and remyelination. To assess the fonction Olig gene in human CNS development we analysed the effects of oligodendrocyte forced expression in human neural stem cells. Neural stem cells were derived from 8 to 10 weeks old human foetuses and grown as neurospheres in FGF2+EGF supplemented medium. The low transfection yield obtained with neural stem cells requires an extensive selection of stable transfected clones which may affect the multipotency of the neurospheres. However HIV-derived lentiviral vectors infect neural stem cells with high efficiency. Therefore, we designed two lentiviral vectors encoding for Olig1 and Olig2. This lentiviral constrcts were tested in human neurospheres by classical transfection protocols. Our preliminary da
ta showed reasonable transfection effeciency of human neurospheres. Most of the cells which over-expressed Olig2 were stained for A2B5. Quantification of A2B5+ cells showed that over-expression of Olig2 promote the generation of oligodendrocyte progenitors. In order to verify this result in vivo, we will graft human embryonic neural stem cells over-expressing Olig gene in MBP-deficient shiverer mouse brain. This result may lead to the development of novel therapeutical strategies to promote the generation of oligodendrocyte for myelin repair.

ENZYME REPLACEMENT IN A MOUSE MODEL OF METACHROMATIC LEUKODYSTROPHY: THERAPEUTIC EFFICACY AND SIDE EFFECTS

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1Institut für Physiologische Chemie, Rheinische Friedrich-Wilhelms Universität, Bonn, Germany, D-53115 2Anatomisches Institut, Christian-Albrechts-Universität, Kiel, Germany, D-24043 3Zymenex A/S, Hillerød, Denmark, DK-3400 Metachromatic leukodystrophy (MLD) is a lysosomal storage disease which is caused by a deficiency of the lysosomal enzyme arylsulfatase A (ASA). Similar to MLD patients, ASA knock out mice develop neurological symptoms and accumulate the ASA substrate sulfatide in the nervous system and kidney. Repeated intravenous injection of recombinant human ASA (rhASA) into ASA-deficient mice reduces sulfatide storage and improves peripheral nerve conduction and rotarod performance. Treatment, however, also leads to the development of increasing titers of anti-hASA antibodies and immune-mediated side effects. First, progressive hypersensitivity reactions causing anaphylaxis and high mortality. Second, resistance to treatment resulting in the reaccumulation of initially reduced sulfatide levels. Serum from seroconverted mice blocked sulfatide hydrolysis also in cell culture systems of MLD, but did not contain antibodies which inhibit ASA activity directly. Cell culture experiments revealed that preincubation of rhASA with mouse immune serum increases the internalization rate by a mannose 6-phosphate (M6P)-independent pathway and diminishes the intracellular half life of endocytosed ASA. The neutralizing effect therefore seems to be due to antibodies which retarget the substitut ed enzyme from M6P receptors to other endocytic receptor systems.

Most human MLD patients do express residual levels of ASA which are likely to prevent severe immune-mediated side effects. To test this notion and to generate an improved animal model of MLD, we generated ASA knock out mice which express an inactive hASA mutant from a stably integrated transgene. First data indicate that these mice are indead immune tolerant and allow for long-term treatment with rhASA.

MASS SPECTROMETRIC QUANTIFICATION OF SULFATIDES IN MLD LEUKODYSTROPHIES

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Sulfatides are a component of myelin and some non-neuronal cells that are particularly over excreted in disorders such as metachromatic leukodystrophy (MLD). Thus, their quantification is a crucial step for MLD diagnosis and prognosis. Physiological levels in urine are comprised between 30 and 190 nM, while pathological levels are comprised between 30 and 3000 nM. Sulfatides quantification is usually performed in urine samples based on a liquid-liquid extraction followed by mass spectrometric analysis. However, despite its medical relevance, we could find only one report relating sulfatides quantification in serum. Patient convenience, stability of matrix composition and reproducibility are the main reasons that convinced us of the needs for a polyvalent sulfatides assay. To this end, a new sample preparation strategy was developed for sulfatides quantification in various body fluids including plasma and urine. This protocol is named "one-pot PP?SPE" (Protein Precipitation-Solid Phase Extraction) because it further allows to perform two extractions in one simple step and more over in a single container. Final quantitative measurements are then performed by mass spectrometry using either ESI-MS or MALDI-TOF-MS in negative ionisation mode. The lower limits of detection and quantification (LLOD and LLOQ) achieved so far for sulfatides in biological samples are of 50 nM and 500 nM, respectively. This new assay is efficient, rapid and its major advantage is that it is applicable to the quantification in both plasma and urine samples. Preliminary data on human pathological samples will be presented.

AGGREGATES OF MUTATED GFAP FORM AGGRESOMES OR DISAGGREGATE IN AN ASTROCYTIC MODEL OF ALEXANDER DISEASE

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Alexander disease (AXD)—a rare neurodegenerative disorder characterized by large cytoplasmic aggregates in astrocytes and myelin abnormalities—is caused by dominant mutations in the gene encoding glial fibrillary acidic protein (GFAP), the main intermediate filament (IF) protein in astrocytes. We tested the effects of several mutations associated with AXD, as well as putative non-pathogenic polymorphisms of the GFAP gene, in cells transiently expressing GFAP fused to green fluorescent protein (GFP). The human SW13 cells (deficient in IF proteins) and mouse astrocytes were used as in vitro models:

- As expected, the four GFAP polymorphisms tested (D292N, P44L, V112I, E220Q, and D154N) behave as wild type GFAP, forming essentially an apparently normal network in astrocytes as well as in SW13 cells.
- 4 published pathogenic mutations located in the rod domain (R239H, R79H, V87I, L235P) behave similarly, forming a network, aggregates and network, or only aggregates in astrocytes; and only aggregates in SW13 cells. However, R416W, located in the tail domain forms only a network and never aggregates, both cell types.
- 2 new mutations located in the tail-domain, R406 and T412, behave differently from rod-domain mutations (and from R416W mutation), mostly forming very small and diffuse aggregates, disrupting the endogenous network. These results indicate a particular effect of mutations in this part of the molecule, implying interaction of the tail domain with another domain of GFAP, or with other unknown protein partners; whereas mutations affecting the rod domains are directly involved in an abnormal polymerisation of GFAP monomers.

In astrocytes from wild-type, GFAP-, and vimentin-deficient mice, mutated GFAP-GFP (R236H and R79H) may aggregate or form a network, depending on qualitative and quantitative interactions with normal IF partners. Interestingly, vimentin revealed chaperone-like molecule properties allowing formation of a normal network with mutated GFAP.

Using a proteasome inhibitor, the ubiquitination of aggregates was highlighted, indicating an effort to eliminate abnormally folded proteins by the ubiquitine-protéasome system, and suggesting that pathophysiological mechanisms involved in other neurodegenerative disorders related to protein aggregation contribute also to AXD.

Time-lapse recordings of living astrocytes showed that aggregates of mutated GFAP-GFP coalesced into aggresomes associated with cell death or disappeared, which was associated with astrocyte survival. Since aggregation of mutated GFAP was dynamic and reversible, therapeutic approaches may be possible, we are currently testing one of them (geldanamycin).

Our research project comprises the following steps:

- Proteomics and peptidomic approaches (MALDI-TOFF, coll. with R. Stocklin, Atheris Laboratories, Geneva, Switzerland; 2D electrophoreses and/or nano LC MS/MS Post-Genomique Platform (P3S), University Pierre et Marie Curie de Paris 6.
- Inhibition of astrocyte death using XIAP (coll. with S. Gandhour, CNRS Strasbourg)
- Targeting mutated GFAP mRNAs by RNA interference (Collaboration with J. Mallet et A. Privat, Inserm, Paris, Montpellier)

- Generation of an AXD in vivo model: Knock-in mice expressing GFAP mutations (GIS-ICS)

Our results could have potentially therapeutic applications for AXD as well as for other leukodystrophies linked to a primary defect in astrocytes such as *MLC1* and *EIF2B* related diseases. They could also be useful in assessing the role of the non-myelinating glial cells in the pathophysiology of other leukodystrophies, as well as in other situations such as energy metabolism impairment, CNS injuries, infections or abnormal protein accumulation.

NEWBORN SCREENING FOR X-LINKED ADRENOLEUKODYSTROPHY (X-ALD) AND OTHER PEROXISOMAL DISORDERS

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Identification of boys with X-ALD in the newborn period before they develop symptoms would make it possible to prevent morbidity and death from Addison Disease due to X-ALD and reduce the risk for the childhood cerebral phenotype by dietary therapy and, when indicated, timely provision of hematopoietic transplant, and also increase the likelihood of success of future therapies,but no method suitable for mass screening is available. Utilizing combined liquid chromatography-tandem mass spectrometry as the analytical method we have demonstrated a ten-fold excess of lysophophatidyl choline containing hexacosanoic acid (C26:0) in dried blood spots on a filter paper matrix from 25 male patients with X-ALD compared to 19 controls. A sixty fold increase was present in 9 subjects with disorders of peroxisome biogenesis. There was no overlap between normal subjects and affected individuals. Studies on a larger series of samples are in progress. A high throughput procedure that wiil permit analysis of 700 samples a day is being developed. A pilot study of 60 000 newborn samples that can be added to existing newborn screening programs is planned.

WHITE MATTER TRACT INJURY AFTER FOCAL SEIZURES IN MICE

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Hippocampal sclerosis is the commonest lesion identified in temporal lobe epilepsy and has been proposed as cause and effect of seizures. An emerging concept in epilepsy research is that the propagation of paroxysmal activity is necessary and sufficient to transform a naïve structure into one capable of generating spontaneous seizures. This transformation underlies an epileptogenic focus which entrain the generation of seizures in interconnected structures. We evaluated white matter tract injury within the corpus callosum in a seizure model in mice. Adult male C57Bl/6 mice underwent focally-evoked seizures induced by intraamygdala kainic acid microinjection with continuous electroencephalography. Seizures were terminated by lorazepam (i.v), 40 or 50 minutes following kainate/vehicle injection. 24h later, coronal sections were processed for TUNEL. Seizures in mice terminated after 40 min resulted predominantly in unilateral TUNEL staining within ipsilateral CA3 and CA1. In con trast, when seizure activity was extended to 50 min contralateral CA1 and CA3 hippocampal damage was more frequently observed. Examination of corpus callosum revealed the presence of cell death. Our data also revealed significant correlation between numbers of degenerating cells in contralateral corpus callosum and the severity of neuronal cell death in CA3 hippocampus ipsilateral. These data establish that prolonged focal limbic seizures in mice induce damage to non-neuronal elements within the corpus callosum and show the degree of this injury is a function of hippocampal damage. Our data indicate seizures injure white matter tracts and offer a potential mechanism for seizure generation and spread to contralateral and extrahippocampal structures.

MOLECULAR CHARACTERIZATION, IN A MODEL SYSTEM, OF THE GENETIC RELATIONSHIP BETWEEN pABC1 (ALDP ORTHOLOG) AND PEX2

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Peroxisomes are involved in numerous metabolic functions. Their deficiencies generally lead to severe diseases such as Zellweger Syndrome (due to defective peroxisome biogenesis) or Adrenoleukodystorphy (due to a deficiency of peroxisomal ABC transporter ALDP). Podospora anserina is a suitable organism to study peroxisome involvement in development. The sexual reproduction of this fungus is blocked in absence of PEX2, a peroxin responsible for Zellweger Syndrome in humans. This defect could be corrected by restoring peroxisome biogenesis upon overproduction of pABC1 (ALDP ortholog)1, as in mammals, or in the presence of certain suppressing mutations (suo5 and suo6)2. We are interested in understanding the relationship between pABC1 and PEX2 and in characterizing the suo genes. PEX2 is part of a protein complex (RING-finger complex) required for the import of peroxisomal-matrix proteins. We have deleted the other members of this complex, PEX10 and PEX12, and shown that PEX2 involvement during development can be extended to the RING-finger complex: absence of either produce the same defects. Interestingly, contrary to PEX2, developmental defects generated in absence of these proteins could not be restored by pABC1 overproduction, furher experiments to specify the interactions between these proteins are in progress. Additionally, we also found that a mutant strain completely devoid of the RING-finger complex can be, at least partially, suppressed by both suo mutations. We have also narrowed suo6 location to a cosmid-containing sequence. Its further characterization should provide alternative ways to correct peroxisomal deficiencies. 1Boisnard, et al, 2003. Mol Microbiol, 49:1287. 2Ruprich-Robert, et al, 2002. Genetics, 161:1089.

P4M: THE PROTEOMICS FOR MYELIN MASS SPECTROMETRY PLATFORM

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Adrenoleukodystophy (ALD), metachromatic leukodystrophy (MLD) and childhood ataxia with central hypomyelination / leukoencephalopathy with vanishing white matter (CACH/VWM) are major forms of leukodystrophies affecting the brain white matter that are characterized by marked phenotype variability. Despite the crucial importance of new therapeutic approaches that are currently developed, no specific biomarker has been described to help in predicting the phenotypic variability and the disease severity. To this end, the „P4M‰ - Proteomics for Myelin - platform was recently created and the acquisition of two high technology mass spectrometers was co-funded by ELA. P4M is dedicated to projects oriented towards the discovery, characterization, validation and quantification of biomarkers for human diseases linked to orphan myelin disorders. During its first year of existence, reliable analytical methods were first developed and validated on healthy brain and plasma samples. The optimization of sample preparation steps and mass fingerprinting by LC-ESI-MS, off-line MALDI-TOF-MS analyses and de novo MS/MS sequencing was successfully achieved. More than 2000 compounds can now routinely be detected in human white matter and more than 1000 compounds in plasma. This methodology is presently applied to pathological plasma samples originating from ALD and CACH/VWM patients. The primary objectives include the identification of specific biomarkers of these pathologies, and preliminary data will be presented. The identification of biomarkers is not only expected to lead to reliable diagnosis and prognosis tools in order to facilitate clinical decisions. It should also provide a better understanding of leukodystrophies and possibly assist the design of novel therapeutic approaches.

MIGRATION POTENTIAL OF NEURAL STEM CELLS OF THE SUBVENTRICULAR ZONE : IMPLICATION OF SLIT/ROBO PROTEINS.

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The subventricular zone (SVZ) of the lateral ventricles is a germinative area of the adult mammal CNS. SVZ cells are stem cells retaining the capacity to migrate over long distances along the rostral migratory stream (RMS) to replace neurons of the olfactory bulb. In response to experimental demyelinations, these cells are able to deviate from the RMS toward lesions and to differentiate into oligodendrocytes. Study of factors responsible for the migratory properties of the SVZ cells led to the identification of Slit proteins. Slits are a family of chemo-repellent factors first identified in the septum and the choroids plexus of mammals. They act on the SVZ cells via a gradient of concentration. These proteins bind to receptors of the roundabout (Robo) family. A precedent work performed in our laboratory highlighted the expression of Slit/Robo proteins in the septum and in the RMS, suggesting they may play an important role in this particular cell migration. In this study, we investigated the function of Slit and Robo proteins using the neurosphere model. Our previous results showed that Slit1 was implicated in the direction and the mode of migration of the SVZ cells. Here we present different experiments that may confirm the implication of Slit1 in the phenomenon of chain migration. In addition, we performed preliminary studies aiming to identify the putative receptor of Slit1 . SVZ cells are interesting targets for therapeutic strategies. Study of mechanisms responsible for their mode of migration is of importance in order to improve cell replacement.

TARGETING OF PERIPHERAL NERVOUS SYSTEM WITH INTRAMUSCULAR GENE THERAPY IN MLD MOUSE

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Metachromatic leukodystrophy (MLD) is an autosomal recessive disorder caused by the deficiency of arysulfatase A (ARSA) leading to lysosomal storage of cerebroside-3-sulfate (sulfatide) and subsequent progressive demyelination within the central and peripheral nervous system. The lack of treatment in late infantile MLD urges new therapeutic approaches.
We demonstrated previously that direct intracerebral targeting of the normal ARSA gene through injection of an adeno-associated vector (AAV5/ARSA) is able to prevent or improve most of the biochemical, neuropathological and clinical defects of ARSA knockout mice. We are currently evaluating both AAV1 injection and plasmid electrotransfer in muscle to target ARSA to the peripheral nerve, possibly through the uptake of ARSA enzyme at the neuro-muscular junctions, its retrograde transport along axons, and even its diffusion in neuronal cells of the spinal cord.
In a first set of experiments, we checked *in vitro* if the addition of the erythropoietin (Epo) secretion signal sequence to the ARSA enzyme led to an increased secretion of the recombinant enzyme, which could enhance the uptake of the recombinant enzyme at the neuromuscular junction. Transfection of both pV/ARSA and pV/ARSA-Epo plasmids in 293T cells led to a significant increase in ARSA activity in transfected cells and mediums. Addition of the Epo secretion signal resulted in a 2-fold increase in ARSA secretion.
Three months old MLD mice were injected in right gastrocnemius and tibialis anterior with either PBS or AAV1/ARSA or plasmids (pV/ARSA and pV/ARSA-Epo) followed by electroporation (200V/cm, 2Hz, 8 pulses of 20 ms). Mice were sacrificed 6 weeks after the injection of AAV1/ARSA and 8 days or 30 days after plasmid electroporation. Injected muscle, sciatic nerve, spinal cord and brain were assessed for ARSA expression and diffusion (ELISA, immunohistochemistry).
Both AAV1 injection and plasmid electroporation induce the expression of the recombinant enzyme in the muscles, ARSA levels being 25-fold higher after AAV1 delivery. Secreted ARSA is recaptured by the peripheral nerve, however in few amounts. Spinal cord analyses are in progress. We confirm that targeting ARSA in muscle is able to allow expression of the recombinant enzyme in the peripheral nerve. In a therapeutic perspective, strategies aiming to enhance retrograde recapture of the enzyme would be necessary to expect a clinical benefit in patients.

EFFECTS OF PATHOLOGICALLY RELEVANT BRANCHED-CHAIN AND VERY LONG CHAIN FATTY ACIDS ON MITOCHONDRIAL PHYSIOLOGY IN RAT BRAIN CELLS AND MITOCHONDRIA

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Accumulation of branched and very long chain unbranched fatty acids caused by genetically defective degradation is characteristic for neurodegenerative diseases like Refsum disease and X-linked adrenoleukodystrophy (X-ALD). Here, the neurodegenerative potency of branched chain phytanic acid (Phyt) found in Refsum disease and very long chain fatty acids (VLCFA) characteristic for X-ALD was investigated in a cell type-specific manner. We quantitatively analysed the effect of Phyt on astrocytes, oligodendrocytes and neurons from rat hippocampus, measuring cytosolic Ca^{2+} level, mitochondrial potential and generation of reactive oxygen species In neurons, Phyt caused an immediate and sustained Ca^{2+} deregulation, mitochondrial depolarization and moderately increased ROS generation. Neurons are more sensitive to Phyt-mediated Ca^{2+} deregulation than astrocytes. Oligodendrocytes cultured together with neurons and astrocytes are much stronger affected by Phyt both with respect to Ca^{2+} deregulation and mitochondrial depolarization. Detailed analysis of mitochondrial physiology was done using isolated mitochondria revealing that phytanoyl-CoA strongly inhibited the mitochondrial 2-oxoglutarate dehydrogenase complex. Direct effects on mitochondrial physiology were shown with cyclodextrin- solubilized C_{24}-fatty acid affecting the mitochondrial potential of isolated liver mitochondria and possibly inducing the opening of the mitochondrial permeability transition pore. Molecular biology studies should clarify the cell physiological changes in cells with a down regulation of the X-ALD-protein and consequently an accumulation of intracellular very long chain fatty acids.

TOWARDS THE FUNCTION OF THE ADRENOLEUKODYSTROPHY-RELATED GENE (*ABCD2*).

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X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disorder due to mutations in the *ABCD1* (*ALD*) gene which encodes for ALDP, a half ABC transporter of the peroxisomal membrane supposed to transport very long chain fatty acids. The *ABCD2* gene which encodes for ALDRP, the closest homolog of ALDP, can compensate for *ABCD1* deficiency when overexpressed. To characterize the function of ALDRP and to better understand this phenomenon of functional redundancy, we have generated stable cell lines expressing an ALDRP-EGFP fusion protein in an inducible manner. The obtained cell lines will be an indispensable tool in our further studies aimed at the resolution of the function of ALDRP. We have hypothetized that ALDRP can be preferentially involved in the peroxisomal metabolism of omega3 very long chain fatty acids. To limit the choice of the putative substrates of ALDRP to test in our cell model, we have analyzed the impact of dietary omega3 fatty acids on the *Abcd2* expression in rats fed an □-linolenic acid-deficient diet effect for two generations. We have extended this study using trangenic mice *Fat-1* able to accumulate omega3 fatty acids.

INTRACEREBRAL AAV5-MEDIATED GENE TRANSFER IN METACHROMATIC LEUKODYSTROPHY: PRECLINICAL DEMONSTRATION OF EFFICIENCY AND TOLERANCE IN MOUSE MODEL AND PRIMATE

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Metachromatic leukodystrophy (MLD) is a neurodegenerative disease caused by deficiency of the lysosomal arylsulfatase A (ARSA). Resulting sulfatide storage in neurons and glial cells leads to neuronal damage and severe demyelination in the central and peripheral nervous systems (CNS, PNS). The most frequent and severe form of MLD (late infantile) starts around 1-2 years, fatal outcome occurring within 2-5 years, without efficient therapy. Enzyme replacement therapy may prevent PNS involvement while CNS correction is possible by the transplantation of genetically modified hematopoeitic cells. Due to the quickness of the CNS neurodegenerative process in patients with rapidly progressive forms of MLD, alternative therapy is needed to allow fast and substantial delivery of the missing enzyme into the brain.

We showed that AAV5-mediated gene transfer in the brain of ARSA knockout mice, that mimics MLD, reverses sulfatide storage and prevents neuropathological abnormalities and neuromotor disabilities when performed at a an early stage of disease (pre-symptomatic, 3 months of age). Similarly, vector delivery performed at a symptomatic stage (6 months of age) reverses sulfatide storage and prevents histopathological abnormalities. However, treated mice continue to develop neuromotor disability, may be due to the lack of correction of biochemical abnormalities involving gangliosides and galactocerebroside.

Although additional initiatives may be required to reverse the course of the disease at a symptomatic stage, AAV5-mediated gene transfer represents a promising strategy to treat patients with rapidly progressive form of MLD. Towards clinical application, we have scaled-up this strategy in primate to determine if a limited number of brain injections is not toxic and allows sufficient expression and diffusion of the recombinant ARSA in a larger size brain, compatible with clinical benefits in patients. Preliminary data will be presented.

POSTNATAL GLIOGENIC ACTIVITIES ARE MODIFIED IN THE RAT CORTEX AFTER HYPOXIA-ISCHEMIA (HI)

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Neurogenesis is nearly completed after birth, whereas gliogenic activities remain intense in the postnatal period. We aimed to study the effects of HI on these critical postnatal processes in the newborn rat parietal cortex (pCx). P3 rats had right carotid ligation and exposure to 6%O2. Glial markers were used: GFAP for astrocytes (AS), NG2 for oligodendrocyte progenitors (OLP), RIP for mature oligodendrocytes (OL). Brdu was injected 24h after HI and brains collected 48h, 72h and 7d after HI (24-48h, 24-72h and 24-7d groups). Another group received Brdu 7d after HI and brain collected at 8d (7d-8d group). Within the ipsilateral and the contralateral pCx, proliferation was measured by counting all double-labelled cells. In the ipsilateral pCx, GFAP and RIP positive cells were increased at all time-points; NG2 positive cells were increased at 48h and 72h. An increased glial proliferation was present in the ipsilateral pCx: GFAP/Brdu astrocytes were increased in all groups; NG2/Brdu OLPs were increased in the 24-48h and 24-72h groups; and RIP/Brdu OLs were increased in the 24h-7d group. In the 7d-8d group, an increase proliferation of all cell types was present in the ipsilateral pCx. These data show significant modifications in the glial architecture of the PCx after mild HI injury, which could contribute to long-term alterations in cerebral development after neonatal injury.

EVALUATION OF THE THERAPEUTIC POTENTIAL OF INTRAVENOUS DELIVERY OF NEURAL STEM CELLS AND MESENCHYMAL STROMAL CELLS IN DEMYELINATED MOUSE MODEL

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Cellular therapy using neural stem cells (NSC) and mesenchymal stromal stem cells (MSC) is under investigation for its therapeutic potential in neurodegenerative disorders, as NSC and MSC may promote and/or participate in regeneration. In this context, we isolated and expanded ex vivo NSC and MSC from the bone marrow and brain, respectively, of adult green fluorescent protein (GFP) transgenic mice and studied their ability to induce remyelination. Two recipient mouse models presenting demyelination were used: the lysolecithin-induced model of focal nerve demyelination and the Twitcher mouse, as a model of multifocal demyelination. NSC were grown as neurospheres and their multipotency was demonstrated through their ability to give rise in vitro to neurons, oligodendrocytes and astrocytes. As MSC cultures are heterogeneous, we started by establishing their molecular signature; MSC used in this study were CD34 and Sca-1 positive but negative for the hematopoietic marker CD45. Intravenous delivery of either NSC or MSC improved functional recovery of lysolecithin-demyelinated mice, as assessed by rotarod performance. Moreover, transplanted mice presented GFP-positive cells in the lysolecithin-demyelinated nerve, indicating that donor MSC and NSC are capable of crossing the blood-nerve barrier. In the demyelinated nerve of NSC transplanted mice, myelin-like GFP positive structures were observed. In relation to Twitcher mice, NSC transplantation increased their lifespan; their nervous system is currently under investigation for the search of GFP positive grafted cells. Further studies are needed to determine the mechanism through which cellular therapy induces functional recovery in demyelination mouse models.

BEHAVIOURAL AND COGNITIVE DYSFUNCTION IN ADULT MLD

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Metachromatic leukodystrophy is biochemically characterized by an accumulation of sulfatides (sulfogalactosylceramides).The deficient enzyme is a lysosomal hydrolase, cerebroside sulfate sulfatase (arylsulfatase A). We have studied 12 adult cases which started by behavioural abnormalities. To evaluate the cognitive and psychiatric status of those patients, we used a series of tests : the criteria of the DSM4 for schizophrenia, the Mini Mental Status, memory tests for encoding and retrieval and a frontal assessment battery. All these patients had disorganized behaviour and social dysfunction which lead to psychiatric departments. Delusions and hallucinations were found in 2 patients. Most of the patients had a frontal lobe syndrome. In all the patients, a cognitive decline was observed. Most of these patients remained for many years without any neurological symptoms, and the diagnosis was only made when Magnetic Resonance Imaging was performed. Arylsulfatase A deficiency and sulfatiduria were present. Genetic studies were performed in 10 patients. Seven patients were compound heterozygotes for the I179S and the infantile 459+1G>A mutation. One patient had the D255H and E312D mutations. For two patients, direct sequencing failed to detect any molecular defect; genomic rearrangements of the gene can be expected.

The vast majority of patients with a late onset and behavioural/cognitive signs at onset had the same genotype highly supporting that genotype strongly influences phenotypical characteristics of the disease. However, as 3 patients with a close phenotype had different genotypes, we have to understand how different combinations of mutations can have the same impact on disease expression and evolution.

EXPLORING THE REQUIREMENT OF CDK2 FOR NORMAL WHITE MATTER DEVELOPMENT AND MYELIN REPAIR FOLLOWING ACQUIRED DEMYELINATION

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Type 2 cyclin-dependent kinase (Cdk2), which controls G1/S transition in eukaryotic cell cycle, was recently shown to be dispensable during embryonic development since Cdk2-null mice develop normally until adulthood. Previous work showed that Cdk2 controls oligodendrocyte progenitors (OPC) cell cycle progression and is downregulated in adult OPCs in vitro. We assessed here the requirement of Cdk2 for proliferation of CNS precursor cells that generate newborn oligodendrocytes in specific regions of the adult brain. We analyzed subcortical white matter, corpus callosum, striatum and cerebellar white matter areas with a broad spectrum of antigenic markers for distinct stages of oligodendroglial maturation (CNPase, MAG, NG2 and Olig2), and found that oligodendroglial lineage development was normal in adult Cdk2-null mice. We next used a model of focal lysolecithin-induced lesion of the corpus callosum in order to challenge the role of Cdk2 in OPC proliferation and oligodendrogenesis following acquired non-autoimmune demyelination. We observed an increase of Ki67+ (cell proliferation marker) cells in the sub-ventricular zone (SVZ) and in the lesion of demyelinated WT mice compared to demyelinated Cdk2-null mice. Altogether, our data provide evidence that Cdk2 does not appear to be essential for normal developmental myelination, suggesting the function of Cdk2 may be effectively compensated in neonatals OPCs. However, it seems to be involved in cell cycle kinetics in adult OPCs following demyelination, and thus could alter myelin repair.

PLP1 OVEREXPRESSION IN PMD AND PMLD PATIENTS: INTEREST OF FIBROBLASTS ANALYSIS

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The *myelin proteolipid protein 1* (*PLP1*), which encodes for the main proteins of the CNS myelin, is implicated in an X-linked disorder of CNS myelination, Pelizaeus-Merzbacher Disease (PMD). Large duplications including the whole gene account for 60% of gene alterations. Transgenic mice with additional *Plp* gene copies are identically hypomyelinated and have demonstrated that *Plp* overexpression may act in a dominant negative effect on myelinating oligodendrocytes that enter into apoptosis. By real time quantitative RT-PCR, we have quantified PLP/DM20 mRNA level in tissues and cells accessible (and known to express *PLP1*) from PMD patients carrying a *PLP1* gene duplication, i.e. nerve biopsies (n=6) and cultured fibroblasts (n=14). An overexpression of the PLP/DM20 transcripts was observed in all samples compared to controls. These findings suggesting that PLP1 expression in fibroblasts and in PNS may reflect PLP1 expression level in the CNS, fibroblasts from 17 male PMD patients without *PLP1* nor *GJA12* genomic abnormality detected (Pelizaeus-Merzbacher Like Disease, PMLD), were analyzed. Among them, 8 overexpressed PLP/DM20 mRNAs implicating that alternative mechanisms to gene duplication could lead to PLP/DM20 overexpression. No genomic mutation nor rearrangement have been found in the *PLP1* promoter and in the ASE *cis* regulating element but one mutation was observed for 1 patient in the *PLP1* gene 3'UTR (its functional relevance on the mRNA stability is under evaluation). Finally, fibroblasts represent a useful tool to quantify the *PLP1* gene expression level and have allowed us to demonstrate that the *PLP1* gene remains a candidate gene for PMLD patients.

CHARACTERIZATION OF NEW TRANSCRIPTS OF THE HUMAN *PLP1* GENE.

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The *myelin proteolipid protein 1* (*PLP1*) gene encodes, by alternative splicing, for two protein isoforms: PLP & DM20. In the CNS, proteolipoproteins are expressed specifically by oligodendrocytes and are integrated in myelin sheets where they participate to the assembly and the stabilization of the compact myelin. An additional exon (exon1.1) localized in mouse *Plp1* intron1 has been described. Spliced between exons 1 and 2, it give rise to transcripts that encode for new protein isoforms corresponding to classic PLP/DM20 proteins with 12 additional amino acids at their N-termini. Although the *PLP1* gene, as well as the encoded proteins, are very conserved among species, mouse exon1.1 is not found in other species. Analyzing the human *PLP1* intron 1 sequence by in silico analysis, we have identified 4 putative exons. Northern Blot and RT-PCR analysis have confirmed the existence of 3 exons and have shown that alternative splicing of these exons generate 5 new transcripts (with both PLP & DM20 mRNAs isoforms). Those new transcripts are human specific and their expression is found preferentially in the CNS, from the fetal development. In situ hybridization has demonstrated that the new transcripts are mainly expressed in neuronal cells from various structures. The proteins encoded by those transcripts are under characterization. At least, two transcripts should produce PLP/DM20 proteins with 9 additional amino acids at their N-terminal parts. Further studies are needed to better understand the functional significance of those new neuronal *PLP1* transcripts which can contribute to the axonal degeneration observed in PLP-related disorders.

MOLECULAR DEFECTS INVOLVED IN PELIZAEUS MERZBACHER LIKE DISEASE (PMLD)

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More than 400 families have been collected in our lab since 1996 with clinical, electrophysiological and MRI suggesting a primitive disorder of myelin formation (PMD, SPG2 related disorders). Out of them, 50% remain without any detected abnormality in the *PLP1* gene (i.e. duplication, deletion or mutation) despite a similar phenotype. They have been named Pelizaeus-Merzbacher Like Disease (PMLD). In only 7% of cases, mutations of *GJA12* have been found. This suggests genetic heterogeneity and/or allelic heterogeneity at the *PLP1* locus with some mutations being undetectable by current screening methods. In order to identify the molecular defects involved in PMLD, we have developed different approaches. Analysis of the *PLP1* gene for other abnormalities has shown that small intragenic rearrangements as well as intronic mutations that could impaired the correct splicing of the PLP/DM20 mRNAs are not involved. Nevertheless, the PLP1 gene still remains a candidate gene since PLP/DM20 mRNAs overexpression was observed in 8/17 PMLD fibroblasts analyzed (see poster *Bonnet Dupeyron et al.*). On the basis of the existence of animal models presenting with a phenotype close to the human pathology and/or functional homologies with the PLP/DM20 proteins, rearrangements in the *MBP* and the *GPM6b* genes have been tested in PMLD patients but no abnormality was found. Other candidates genes, including those presenting homologies with *GJA12* or involved in oligodendrocytes maturation, are under investigation. Finally, a genome wide linkage analysis using large or consanguineous families collected thanks to collaborations has been initiated in the aim to identify new PMLD loci.

ATAXIA, HYPODONTIA AND HYPOMYELINATION: A NOVEL LEUKOENCEPHALOPATHY

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Recently we described a novel leukoencephalopathy in four girls. The children presented with early-onset ataxia and delayed dentition with an abnormal pattern. Cerebral MRI showed hypomyelination and cerebellar atrophy; dental X-ray revealed variable absence of replacement teeth. We present now four more children with an identical clinical and radiological pattern between 3 and 12 years of age. Three of them are boys. None are from consanguineous families. Two children had natal teeth which vanished after several weeks. Dentition was late, deciduous molars were the first teeth to erupt, and deciduous incisors have not yet erupted in all. Dental X-rays show variable absence of replacement teeth. All children have mainly gait ataxia which became evident in the second year of life, but also mild intention and action tremor. Two of them have developed a pyramidal tract lesion. In three, ataxia worsens during minor infections. The children also show delayed language development mainly of expressive language and borderline cognitive abilities. MRI in all children revealed hypomyelination and cerebellar atrophy. Proton MR spectroscopy with quantification of metabolites was performed in one child and showed elevated white matter myo-inositol, as previously described.
As the clinical and radiological picture in these patients is identical, we suggest that this disorder with ataxia, hypodontia and hypomyelination represents a new entity. With the characteristic tooth abnormalities it should be straightforward to identify new patients in order to facilitate the search for the underlying genetic defect.

PLP1 TRIPLICATION: SEVERE CONNATAL FORM OF PELIZAEUS-MERZBACHER DISEASE WITH EPILEPSY

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We report on a boy, the first child of unrelated healthy parents, who presented after birth with hyperexcitability. Muscle tone was increased. In the following weeks, nystagmus became evident. Tendon reflexes could not be elicited. Epilepsy with mainly tonic seizures started at age 5 weeks and was resistant to therapy. MRI revealed no progress of myelination at age 2.5 and 6 months. The tentative clinical diagnosis of Pelizaeus-Merzbacher disease (PMD) was made, but rejected because of severe epilepsy and absent tendon reflexes. The boy died at age 19 months, in 1993, without firm diagnosis. Recently multiplex ligation-dependent probe amplification (MLPA) of the seven exons of the *PLP1* became available for rapid and exact copy number analysis. The test was carried out in the patient's mother as she has two adolescent daughters and revealed the presence of four *PLP1* copies compatible with a triplication of the gene.
PLP1 triplication is rare and has recently been described in four children. One child is known to have carried a *PLP1* quintuplication. Symptoms in these children are more severe than in patients carrying *PLP1* duplications the main cause of classic PMD. Three of these children with higher *PLP1* copy numbers also suffered from severe epilepsy, as our patient did. In boys with a clinical diagnosis of connatal PMD and seizures, *PLP1* triplication should be suspected and quantitative analysis of *PLP1* performed allowing to discriminate reliably between duplication and triplication.

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