

**Untersuchung von Muskelgewebe amyotropher lateraler Sklerose:
Etiologie, Diagnose und therapeutische Aspekte**

Inauguraldissertation

zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der
Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

von

Franck DI SCALA

aus Strasbourg – Frankreich

Frankreich, 2006

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät
auf Antrag von

Dr Jean-Philippe LOEFFLER

Professor Michael PRIMIG

Professor Markus RÜEGG

Basel, den 25. Oktober 2005.

Prof. H.-J. WIRZ
Dekan

TABLE OF CONTENTS

Preface.....	5
Abbreviations	7
Glossary.....	10
Introduction	16
1. Clinical characteristics of ALS.....	17
1.1. General description.....	17
1.2. Epidemiology	17
1.3. Clinical presentation and course.....	20
1.4. ALS diversity	20
1.4.1. The Western Pacific type	22
1.4.2. The classical sporadic form.....	22
1.4.3. The familial type	22
1.5. Diagnosis	22
1.5.1. Clinical diagnosis.....	25
1.5.2. Differential diagnosis	29
1.5.3. Familial ALS diagnosis	29
1.5.4. Early diagnosis and diagnostic delay	32
1.6. Measurements of disease progression	33
2. Clinical management of ALS.....	39
2.1. Preventive therapy	39
2.2. ALS-specific therapies	39
2.3. Palliative care.....	40
3. Mutant SOD1 transgenic mice	41
4. Skeletal muscle in ALS	44
4.1. Skeletal muscle is a potential tool in ALS monitoring and diagnosis	44
4.2. Potential involvement of skeletal muscle in ALS etiology	46
Objectives and organisation of results	49
Results and discussion	51
1. Nogo-A is a potential marker of ALS	53
1.1. Reticulon family.....	53
1.2. Functions of mammalian RTNs.....	55
1.3. RTNs as disease markers.....	57
1.3.1. RTNs in cancer.....	57
1.3.2. RTNs in neurological diseases.....	57

1.3.3.	RTNs in ALS.....	58
1.3.4.	RTNs are linked to differentiation processes	58
Article 1	60
Article 2	61
2.	Uncoupling protein 3 is a potential marker of ALS	63
2.1.	Uncoupling protein family.....	63
2.2.	UCP2 and UCP3 expression is increased in ALS muscle	63
2.3.	UCP3 is a potential modulator of reactive oxygen species metabolism in ALS.....	64
2.4.	UCP3 is a potential mediator of hypermetabolism in ALS	65
Article 3	66
Article 4	67
3.	Expression profiling of skeletal muscle in patients affected by ALS	69
3.1.	The microarray approach.....	69
3.2.	The technology of microarray	69
3.3.	Gene expression experiment.....	74
3.4.	Data quantitation.....	79
3.5.	Data normalization	79
3.6.	Data mining and modelling	80
3.7.	Sample clustering using G3	83
3.8.	Sample clustering using G9	83
3.9.	Data annotation.....	88
3.10.	Microarray expression data validation	91
3.11.	An example of application: retinoic acid metabolism in ALS.....	91
Conclusion and perspectives	97
References	102

Preface

The laboratory *Signalisations Moléculaires et Neurodégénérescence* headed by Dr Jean-Philippe Loeffler focuses its research on the neuronal death process that occurs in neurodegenerative diseases such as amyotrophic lateral sclerosis and Alzheimer's disease. To understand the mechanisms that trigger the neuronal degeneration, studies are performed both directly on patients affected by these diseases and on cellular or animal models that mimic *in vitro* or *in vivo* the neuronal degenerative process.

My PhD work was dedicated to amyotrophic lateral sclerosis (ALS), also called "*maladie de Charcot*" in Europe or "Lou Gehrig's disease" in United-States. ALS is a progressive neurodegenerative disease that attacks nerve cells in the brain and spinal cord, and produces muscle weakness and atrophy. When I joined the team in 2001, there was a general acceptance that ALS was an exclusive motor neuron pathology. Thus, investigations in the lab focused on gene expression changes occurring in the spinal cord. A differential screening approach, performed on a line of transgenic mice that develop an ALS-like syndrome, revealed strong genetic modifications in the spinal cord that already occurred in the absence of any overt symptoms. Interestingly, some of the modulated genes were also regulated in the skeletal muscles of these transgenic mice and were particularly relevant to ALS. These results, together with data from the literature, not only highlighted the importance of studying the skeletal muscle tissue but also presaged an active contribution of this tissue to the disease process. These considerations prompted us to focus our attention on the skeletal muscle tissue in ALS.

Abbreviations

AD:	Alzheimer's disease
ALS:	amyotrophic lateral sclerosis
ALSFRS:	ALS Functional Rating Scale
ALSSS:	ALS Severity Scale
BACE-1:	β -amyloid converting enzyme 1
CNS:	central nervous system
CRABP-I:	retinoic acid binding protein I
CSF:	cerebral spinal fluid
CTWC:	Coupled Two-Way Clustering
EDX:	electrodiagnostic
ER:	endoplasmic reticulum
ESTs:	expressed sequence tags
FALS :	familial ALS
FDG:	2- ¹⁸ F-2-deoxy-D-glucose
FVC:	forced vital capacity
GDNF:	glial cell line-derived neurotrophic factor
GO:	Gene Ontology
IGF-1:	insulin like growth factor-1
KEGG:	Kyoto Encyclopedia of Genes and Genomes
LINGO-1:	LRR and Ig domain-containing, Nogo Receptor-interacting protein
MAS:	Microarray Analysis Suite 5.0 (Affymetrix)
MM:	mismatch
MMT:	manual muscle testing
MRI:	magnetic resonance imaging
MRS:	magnetic resonance spectroscopy
MS:	multiple sclerosis
MVIC:	maximal voluntary isometric contraction
NAA:	N-acetyl aspartate
NgR:	Nogo receptor
NMJ:	neuromuscular junction
OMIM:	Online Mendelian Inheritance in Man™
p75NTR:	p75 neurotrophin receptor
PET:	positron emission tomography
PM:	perfect match

QPCR:	quantitative polymerase chain reaction
RA:	retinoic acid
RALDH-2:	retinaldehyde dehydrogenase-2
RHD:	reticulon homology domain
RMA:	Robust Multichip Average
ROS:	reactive oxygen species
RTN:	reticulon
SALS:	sporadic ALS
SCLC:	small-cell lung carcinoma
SMN:	survival motor neuron
SOD1:	Cu,Zn superoxide dismutase
SPECT:	single photon emission tomography
UCP:	uncoupling protein
VAPB:	vesicle-associated membrane protein B
VEGF:	vascular endothelial growth factor

Glossary

Analysis of variance (ANOVA): a collection of parametric tests that compare means by splitting the overall observed variance into different parts.

Data mining: the interpretation of microarray data towards the understanding of biological processes.

Data quantitation: the translation of the signal intensities from microarrays into numerical values.

Dendogram: a graphical form of representation of the similarities of gene expression.

Expressed Sequence Tags (ESTs): partial cDNA "survey sequences".

Fold approach: in this approach, a gene is declared to be turned "on" or "off", if the average expression level is increased or decreased respectively by a predetermined number of "folds".

GenBank: the National Institutes of Health (NIH) genetic sequence database, an annotated collection of all publicly available DNA sequences. A new release is made every two months. GenBank is part of the International Nucleotide Sequence Database Collaboration, which is comprised of the DNA DataBank of Japan (DDBJ), the European Molecular Biology Laboratory (EMBL), and GenBank at the National Center for Biotechnology Information. These three organizations exchange data on a daily basis.

Gene expression matrix: a tabular arrangement of gene expression profiles.

Gene Expression Omnibus (GEO): a high-throughput gene expression / molecular abundance data repository, as well as a curated, online resource for gene expression data browsing, query and retrieval.

Gene Ontology (GO): a controlled vocabulary for describing genes, structured hierarchically as a directed acyclic graph (DAG), where nodes (or terms) are more general if closer to the root and more specific if closer to the leaf.

GenMAPP: a free computer application designed to visualize gene expression and other genomic data on maps representing biological pathways and groupings of genes.

Heuristic algorithm: two fundamental goals in computer science are finding algorithms with provably good run times and with provably good or optimal solution quality. A heuristic algorithm gives up one or both of these goals; for example, it usually finds pretty good solutions, but there is no proof the solutions could not get arbitrarily bad.

HG-U133 Target Databank: a compilation of probeset annotations and target sequence information for all the probes represented on the human genome HG-U133 A and B arrays.

Hybridization: the process whereby two complementary DNA or RNA strands join to form a double stranded structure.

Kruskal-Wallis test: a non-parametric test used to compare three or more independent groups of sampled data. It uses the ranks of the data rather than their raw values to calculate the statistic.

Kyoto Encyclopedia of Genes and Genomes (KEGG): a suite of databases and associated software, integrating our current knowledge on molecular interaction networks in biological processes ("pathway" database), the information about the universe of genes and proteins ("genes"/"ssdb"/"ko" databases), and the information about the universe of chemical compounds and reactions ("compound"/"glycan"/"reaction" databases).

Microarray: an orderly arrangement of microscopic elements on a planar surface that allows the specific binding of genes or gene products.

Mismatch (MM): a 25-mer oligonucleotide designed to be complementary to a reference sequence except for a single, homomeric base change at the 13th position. Mismatch probes serve as specificity controls when compared to their corresponding Perfect Match probe.

Nonparametric test: a statistical test without the assumption of a particular distribution of the data, also known as a distribution-free test.

Normalization: a procedure that adjusts an average value of an experimental array equal to that of the baseline array so that they can be compared.

Online Mendelian Inheritance in Man™ (OMIM): a catalog of human genes and genetic disorders authored and edited by Dr. Victor A. McKusick and his colleagues at Johns Hopkins and elsewhere, and developed for the World Wide Web by the NCBI. The database contains textual information and references. It also contains copious links to MEDLINE and sequence records in the Entrez system, and links to additional related resources at NCBI and elsewhere.

Parametric test: a statistical test that assumes that the data sampled was from a population that follows a Gaussian or normal distribution.

Pearson correlation test: a measure of the strength of the linear relationship between two variables, using the mean of the data.

Perfect match (PM): a 25-mer oligonucleotide designed to be complementary to a reference sequence.

Permutation test: a special case of randomization tests, i.e. tests that use randomly generated numbers for statistical inference. Randomization tests differ from parametric tests in many aspects, *e. g.* there is no requirement to have random samples from one or more populations, there is no need to assume anything about normality or variance equality, the null hypothesis has nothing to do with parameters, and the test statistic performed is not compared to tabled distributions but to the

results obtained when the data across the groups are repeatedly randomized (the corresponding statistic for each randomization is then calculated).

Probe cell: a single square-shaped feature on an array containing probes with a unique sequence.

Probe pair: two probe cells, designed as a Perfect Match (PM) and its corresponding Mismatch (MM).

Probe set: two probe cells, designed as a Perfect Match (PM) and its corresponding Mismatch (MM).

Probe: the molecule in solution that reacts with the target on the microarray slide.

Reference Sequence (RefSeq): a collection that aims to provide a comprehensive, integrated, non-redundant set of sequences, including genomic DNA, RNA, and protein products, for major research organisms.

Signal: a measure of the relative abundance of a transcript.

Signal threshold approach: in this approach, a signal threshold is determined and genes whose signal is under this threshold are excluded; those low signal intensities that are near to the background levels increase variability or impair reproducibility of the measured ratios between control and experimental samples.

Spearman correlation test: a measure of the strength of the linear relationship between two variables, using the mean of the ranks of the data, *i.e.* the lowest measurement becomes 1, the second lowest 2, and so forth.

Stable gene/sample cluster (in CTWC): a cluster that is statistically significant according to criteria described by Getz et al., 2002.

Target: the molecule attached to a microarray substrate that reacts with the free probe in the solution.

Temperature parameter T (in CTWC): a tunable parameter that controls the resolution of the performed clustering. One starts at $T=0$, with a single cluster that contains all the objects. As T increases, phase transitions take place, and this cluster breaks into several subclusters that reflect the structure of the data. Clusters keep breaking up as T is further increased, until at high enough values of T each object forms its own cluster.

T-test method: any statistical hypothesis test in which the test statistic has a Student's t -distribution if the null hypothesis is true. Student's distribution is a probability distribution that arises in the problem of estimating the mean of a normally distributed population when the sample size is small. The null hypothesis of a t -test always proposes that the two groups do not differ significantly, while the hypothesis of a t -test always proposes that there is a difference.

Unigene: an experimental system for automatically partitioning GenBank sequences into a non-redundant set of gene-oriented clusters. Each UniGene cluster contains sequences that represent a unique gene, as well as related information such as the tissue types in which the gene has been expressed and map location.

Wilcoxon-Mann-Whitney test: a nonparametric test appropriate to the case of two independent samples of observations that are measured at least at an ordinal level, *i.e.* we can at least say, of any two observations, which is the greater. The test assesses whether the degree of overlap between the two observed distributions is less than would be expected by chance, on the null hypothesis that the two samples are drawn from a single population.

This glossary is based on information provided by Affymetrix, Wikipedia and NCBI websites.

Introduction

1. Clinical characteristics of ALS

1.1. General description

Amyotrophic lateral sclerosis (ALS) is the most common degenerative disorder of the motoneuronal system occurring in adult life. It was first described in 1869 by the french neurologist Jean-Martin Charcot (Charcot, 1874), who named it according to the clinical picture (muscle wasting = "amyotrophy") and the pathological findings (hardening of at least one side of the spinal cord = "lateral sclerosis"). Lou Gehrig brought first international attention to the disease in 1939 when he abruptly retired from baseball after being diagnosed with ALS.

ALS affects motor neurons that reach from the brain to the spinal cord and from the spinal cord to the muscles throughout the body (Fig. 1). As motor neurons degenerate, they can no longer send impulses to the muscle fibers that normally result in muscle movement, so that the ability of the brain to initiate and control muscle movement is lost. For the vast majority of patients, their minds remain unaffected. While there is not a cure today that halts or reverses ALS, there is one Food and Drug Administration approved drug, riluzole (Rilutek®), that modestly slows the progression of ALS.

1.2. Epidemiology

The incidence of ALS has been estimated as around 1.5-2 per 100,000 per year (3-5 for multiple sclerosis (MS)), with a tendency toward an increasing incidence reported (Lilienfeld et al., 1989). Prevalence studies showed that up to 25,000 Western Europeans suffer from ALS at any given time. Most people who develop ALS are between the ages of 40 and 70, with an average age of 58 (Li et al., 1990) at the time of diagnosis. However, cases of the disease do occur in persons in their twenties and thirties. The incidence of ALS seems to increase with advancing age (Juergens et al., 1980).

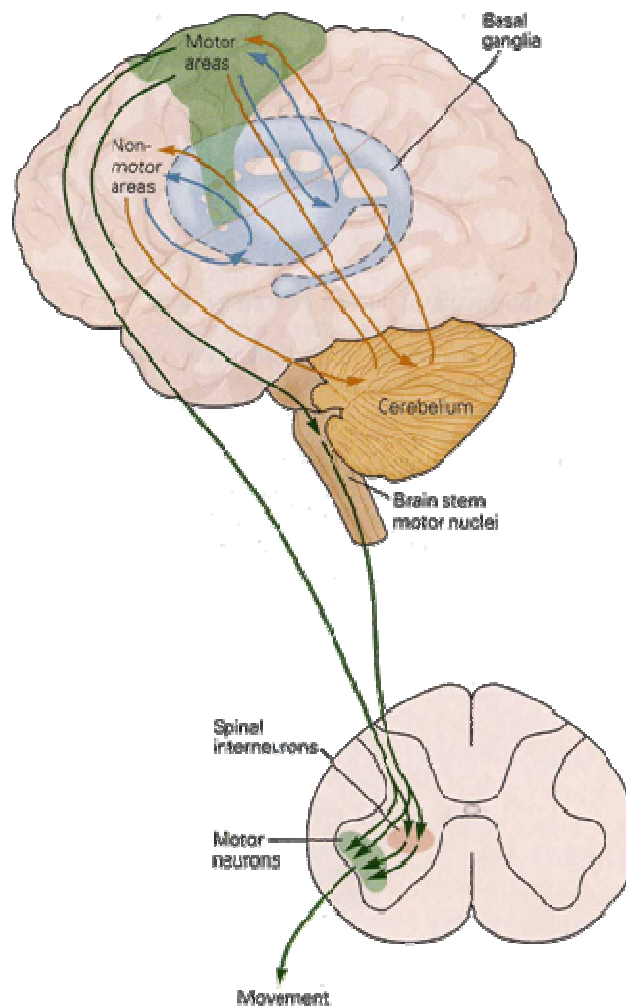


Image courtesy of McGraw-Hill Companies, from Kandel et al.

Fig. 1. Hierarchical organization of the motor system.

The motor system has three levels of control: the spinal cord, the brain stem and the forebrain. The motor areas of the cerebral cortex can influence the spinal cord either directly or through the brain stem. All three levels of the motor system receive sensory inputs and are also under influence of the basal ganglia and the cerebellum.

Geographical differences in incidence have not been reported for ALS, with the exception of high-incidence foci in Asia and Oceania, in three Western Pacific population groups. These included the Chamorro people in Mariana Islands (Guam and Rota) (Reed et al., 1975; Reed & Brody, 1975), the Auyu and Jakai people of West New Guinea (Gajdusek & Salazar, 1982) and the Japanese residents of the Kii peninsula (Honshu island) (Kusui, 1962). The declining annual incidence (Garruto et al., 1985) coupled with the absence of demonstrable heritable or transmissible factors (Figlewicz et al., 1994) had led to focus the search for the cause of these high incidences on nontransmissible environmental factors. Among them, heavy use of certain toxic plants, notably cycads, has been proposed (Spencer et al., 1987), as well as secondary hyperparathyroidism, resulting from low environmental calcium and magnesium, and leading to abnormal deposition of calcium and aluminum in the central nervous system (CNS) (Garruto et al., 1985).

Research studies investigating risk factors that may be associated with ALS proposed smoking, trauma, physical activity, exposure to lead, residence in rural areas and alcohol consumption being a probable risk factor for ALS (Armon 2003 for review). More work is needed to conclusively determine what environmental factors contribute to developing ALS. In contrast, variations in several genes relevant to motor neuron biology have been considered as possible modifying or risk factors for ALS. One example is the survival motor neuron (SMN) gene. Deficiency of the telomeric but not the centromeric copy of SMN gene causes recessively inherited spinal muscular atrophy. Detailed analyses of SMN genes in ALS documented that some variants in SMN seem to modify clinical parameters in sporadic ALS cases (Jackson et al., 1996; Parboosingh et al., 1999; Moulard et al., 1998; Corcia et al., 2002). Polymorphisms or variants in other genes have also been considered as risk factors for ALS, including apolipoprotein E (Mui et al., 1995; al-Chalabi et al., 1996; Moulard et al., 1996; Smith et al., 1996), ciliary neurotrophic factor (Takahashi et al., 1994; Takahashi, 1995; Orrell et al., 1995; Masu et al., 1993; Al-Chalabi et al., 2003), and the astrocytic glutamate transporter EAAT2/GLT1 (Lin et al., 1998; Trotti et al., 1999). Recently, the possibility of a genetic predisposition in ALS was highlighted by a report of a high rate of constitutional chromosomal aberrations (5.9% vs 0.5-0.1% in the general population) (Meyer et al., 2003).

1.3. Clinical presentation and course

The clinical hallmark of ALS is the coexistence of muscle atrophy, weakness, fasciculations, and cramps, together with hyperactive or inappropriately brisk deep tendon reflexes, pyramidal tract signs, and increased muscle tone (Table 1). Muscle cramps are often already present before other symptoms develop. Most patients present with asymmetrical, distal weakness of the arm or leg (Norris et al., 1993). Bulbar onset with slurred speech (dysarthria) and/or difficulty in swallowing (dysphagia) occurs in 20-30% of all cases, particularly in older females, more than 50% of them presenting bulbar symptoms (Li et al., 1990).

The symptoms usually progress first in the affected extremity, then gradually spread to adjacent muscle groups, with a remarkable variability in the rate of disease progression (Appel et al., 1987). Average disease duration is in the range of 3-4 years, with 10% of patients surviving more than 10 years (Mulder & Howard, 1976) and some cases surviving over several decades (Grohne et al., 2001). Respiratory weakness due to high cervical and thoracic spinal cord involvement is the most common cause of death in ALS.

There is usually no clinical involvement of parts of the CNS other than the motor pathways. Sensation, bladder, bowel and eye functions remain intact, and intellectual function together with personality are usually preserved, with only 2-3% of patients showing an associated dementia.

1.4. ALS diversity

Multiple clinical variants of ALS are now recognized which are associated with a spectrum of clinical outcomes from aggressive to rather indolent. Three major types of ALS are generally accepted.

Table 1. Symptoms due to ALS

Direct symptoms	Indirect symptoms	Absent symptoms
Motor weakness	Psychic disturbances	Sensation troubles
Amyotrophy	Sleep disturbances	Extraocular muscles paralysis
Fasciculations	Constipation	Incontinence
Muscle cramps	Drooling	Dementia
Spasticity	Thick mucous secretions	
Dysarthria	Symptoms of chronic hypoventilation	
Dysphagia	Pain	
Dyspnea		
Pathologic laughing & crying		

1.4.1. The Western Pacific type

Often associated with dementia and a Parkinson's syndrome, this ALS form was observed in Guam and the Trust Territories of the Pacific in the 1950's (Brody et al., 1975), with a remarkable concentration since approximately 1 in 10 Guamanian deaths over age 25 was from ALS.

1.4.2. The classical sporadic form

Sporadic ALS (SALS) accounts for 90 to 95% of all cases. For these patients, there is no previous family history of this disorder. What is not clear is what factors contribute to the causation of this form. Although the cause may not be genetic, some mutations are thought to at least contribute to the pathogenesis of sporadic ALS, including deletions in the heavy neurofilament subunit tail (Al-Chalabi et al., 1999) and mutations of mitochondrial DNA (Vielhaber et al., 2000).

1.4.3. The familial type

Familial ALS (FALS) accounts for a very small number of patients with 5 to 10% of all cases. Most of them show an autosomal dominant inheritance pattern. In those families, there is a 50% chance each offspring will inherit the gene mutation and may develop the disease. To date, nine loci have been associated with FALS and mutations in at least four genes have been found to be associated with this disease.

Als1: a major breakthrough in deciphering the molecular mechanisms underlying ALS was provided by the observation that mutations in the gene encoding for the antioxidant enzyme Cu,Zn superoxide dismutase (SOD1) are carried by one-fifth of FALS patients (Rosen, 1993). More than 100 mutations in this gene have been found in familial and sporadic cases of ALS (<http://www.alsod.org>). They are distributed among all five exons of the gene and result in the alteration of amino acids scattered throughout the SOD1 structure (Fig. 2).

Als2: als2 or alsin mutations cause an autosomal recessive form of juvenile ALS and have been mapped to chromosome 2q33 (Hadano et al., 2001; Yang et al., 2001). Alsin long form belongs to the family of the guanine nucleotide exchanging factor for small GTPases.

Als4: mutations in this gene are associated with a rare juvenile autosomal dominant disorder with slow progression, pyramidal signs, and severe muscle wasting (Rabin et al., 1999). This gene encodes a novel DNA/RNA helicase (Chen et al., 2004).

Als8: a missense mutation in this gene causes an autosomal dominant slowly progressive disorder characterized by fasciculation, cramps, and postural tremor. This gene encodes the vesicle-associated membrane protein/synaptobrevin-associated membrane protein B, an intracellular membrane protein that can associate with microtubules and that may have a function in membrane transport (Nishimura et al., 2004).

The clinical expression of sporadic and familial ALS is very similar, although the age of onset is often lower for FALS (Camu et al., 1999), and more men are affected by SALS than women (ratio men/women for SALS \approx 1.7/1 vs 1/1 for FALS). Thus, there is justified hope that insights gained from the study of the mechanisms leading to motor neuron degeneration from SOD1 mutation may also be relevant for SALS.

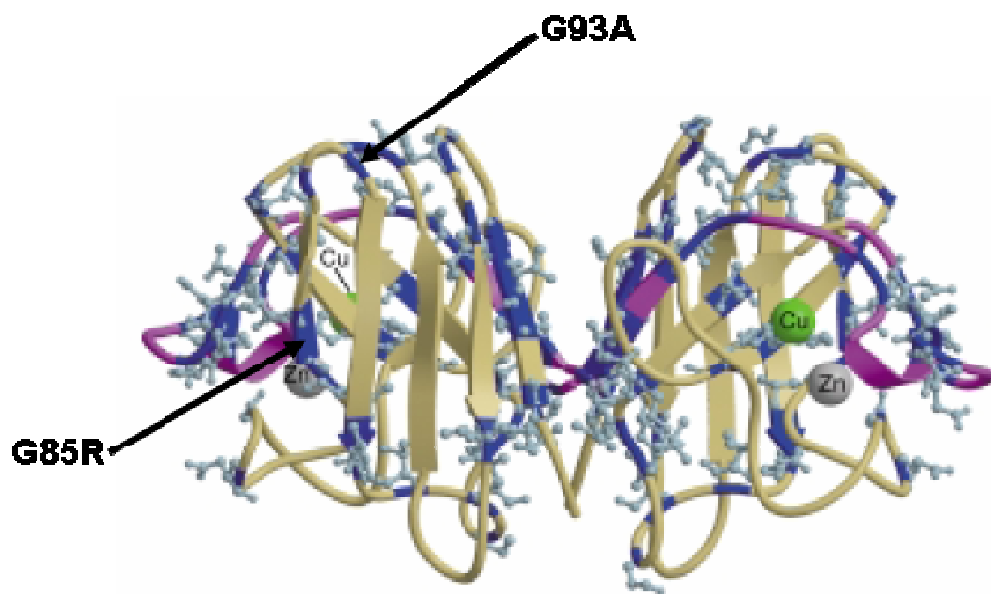


Fig. 2. SOD1 protein is a homodimer (From Beckman et al., 2001).

The sidechains of mutated amino acids are shown in **light blue** and on the backbone as **dark blue**. The majority of the mutations are clustered on the top and bottom of the β barrel, in the dimer interface or along one of the loops that forms part of the Zn-binding pocket. A major part of the C-terminal region shown in **purple** can be entirely deleted in a few individuals. Mutations Gly85Arg and Gly93Ala are indicated by arrows.

1.5. Diagnosis

1.5.1. Clinical diagnosis

The full-blown picture of classical ALS is difficult to misdiagnose, but diagnosis may be difficult at early stages when the patients complain of only unilateral, mostly distal weakness and wasting. Essentially, the diagnosis of ALS is a clinical one, based on criteria established on the basis of international expert consensus (Brooks, 1994) and revised during a consensus conference held at Airlie House, Virginia (Brooks et al., 1998). These so-called El Escorial criteria (Table 2) reflect a consensus agreement concerning those features that imply certainty regarding the diagnosis ("definite ALS"), or degrees of uncertainty ("probable ALS"). The diagnosis of ALS requires the presence of both upper and lower motor neuron signs, with a progression of symptoms and signs over time. Initially devised for research, especially for clinical trials of potential therapies in the disease, the El Escorial criteria have shown some limits for accurate everyday clinical diagnosis. First, sensitivity and specificity of these criteria in relation to autopsy-confirmed experience has not been tested. Second, these definitions of different levels of diagnostic certainty do not indicate neither severity of disease nor rate of progression. Indeed, it is recognized that ALS progresses at different rates and in different ways in individual patients. Third, these criteria were concerned principally with defining classical Charcot's ALS, the most common form of the disease. Indeed, the issue of the relationships of classical Charcot's ALS syndrome with other clinical forms of the motor neuron disease syndrome, especially progressive muscular atrophy, progressive bulbar palsy and primary lateral sclerosis, is not fully addressed.

Identification of upper motor neuron affection is essentially based on clinical evidences, including brisk deep tendon reflexes (Hoffmann or Babinski signs) and spasticity. Structural magnetic resonance imaging (MRI) has proved to be useful in the visualisation of corticospinal tract abnormalities in ALS patients (Cheung et al.,

Table 2. Revised El Escorial criteria

Main features	
UMN signs	pathologic spread of reflexes, clonus...
LMN signs	weakness, atrophy, fasciculations
Progression	
Diagnostic categories	
Definite ALS	UMN plus LMN signs in three regions
Probable ALS	UMN plus LMN signs in at least two regions, with UMN signs rostral to LMN signs
Probable ALS	UMN and LMN signs in one region,
Laboratory supported	or UMN signs in one region and LMN signs defined by EMG criteria in at least two limbs
Possible ALS	UMN plus LMN signs in one region, or UMN signs in two or three regions, or LMN signs in one region rostral to UMN signs
Absence of	
Sensory signs	
Autonomic dysfunction	
Sphincter disturbance	
Parkinsonism	
Alzheimer-type dementia	
ALS "mimic" syndromes	

UMN: upper motor neurons. LMN: lower motor neurons. EMG: electromyography. Regions are defined as bulbar, cervical, thoracic, and lumbosacral.

1995; Mirowitz et al., 1989). Since these changes are non-specific and cannot be readily quantified, MRI essentially serves the purpose of ruling out other causes of the syndrome. However, proton magnetic resonance spectroscopy (MRS), which enables the investigation of metabolic changes *in vivo*, has been widely applied in ALS. Using MRS, different metabolites can be detected in the motor cortex and brainstem, including the neuronal marker N-acetyl aspartate (NAA), creatine / phosphocreatine, choline, myo-inositol, and glutamine / glutamate. The NAA signal has been shown to be decreased both in the motor area and in the brainstem in ALS, such a decrease being dependant on time and progression (Pioro et al., 1994; Block et al., 1998; Cwik et al., 1998). Quantification of NAA may therefore allow the assessment of disease progression but less likely contributes to early diagnosis in ALS, due to unresolved issues in the application of MRS including low sensitivity with substantial overlap between ALS patients and healthy controls, and multiple data acquisition techniques clearly limiting comparison between published studies. Positron emission tomography (PET) is another imaging tool to examine metabolic changes *in vivo*. This technique has been applied to ALS, using 2-¹⁸F-2-deoxy-D-glucose (FDG) (Dalakas et al., 1987; Hatazawa et al., 1988) and ¹¹C-flumazenil, a GABA_A ligand (Lloyd et al., 2000), but further studies on sensitivity and specificity of these methods are necessary to establish ¹¹C-flumazenil or FDG PET as a suitable diagnostic or progression marker.

Lower motor neuron affection is clinically associated with progressive motor weakness, amyotrophy, fasciculations and muscle cramps. In addition to clinical examination, electrodiagnostic (EDX) techniques have been used in the evaluation of patients with clinically suspected ALS for nearly 50 years (Huddleston et al., 1950; Marinacci, 1955). The utility of the EDX examination in the assessment of patients with ALS was firmly established by Lambert (Lambert & Mulder, 1957), who listed a combination of four EDX findings that he considered highly supportive of the clinical diagnosis of ALS : two of these concerned the nerve conduction studies, and other two the needle electrode examination (Table 3). New EDX criteria were formulated by the El Escorial group, but they contain several flaws, which limit their use by electrodiagnostic physicians (Wilbourn, 1998).

**Table 3. The Lambert criteria for the electrophysiological confirmation of ALS
(From Wilbourn, 1998)**

Nerve conduction studies	Needle electrode examination
1. Normal sensory NCS	3. Fibrillation and fasciculation potentials in muscles of the upper and lower extremities or in the muscles of the extremities and the head
2. Motor NCS CVs: * normal when recording from relatively unaffected muscles * not less than 70% of the age-based average normal value when recording from severely affected muscles	4. MUPs reduced in number and increased in duration and amplitude

NCS: nerve conduction studies. CVs: conduction velocities. MUPs: motor unit potentials.

1.5.2. Differential diagnosis

Additional investigations may be required at early stages to exclude treatable diseases, and several diseases must be taken into consideration in the differential diagnosis of early ALS (Table 4). Among them, immune disorders (inclusion body myositis), infections (Prion disease), metabolic disorders (hyperthyroidism) and other neurological disorders (motor neuropathy, myopathic syndromes) may mimic ALS. There is no consensus about the appropriate range of laboratory tests to be ordered in making differential diagnosis of ALS (Table 5). They include cerebral spinal fluid (CSF) examination (the protein content of which seems to be related to lymphoma), bone marrow biopsy (reserved for patients with clinical suspicion of lymphoproliferative disease), quantitative electromyography (to avoid erroneous "neurogenic" patterns) and muscle biopsy (to exclude inclusion body myositis).

1.5.3. Familial ALS diagnosis

Diagnosis can be achieved by genetic techniques in patients with familial ALS and mutations in the gene encoding for SOD1. In families with known SOD1 mutations, mutational screening in persons at risk has the potential to detect preclinical cases and therefore be considered as a true diagnostic marker with high sensitivity and specificity. However, more and more mutations in the SOD1 gene are identified and several genes leading to ALS or predisposing to the disease are progressively discovered. The rarity of these families argues against a major impact of the corresponding gene product as a diagnostic marker. Moreover, a particular ethical dilemma is raised by the availability of these genetic markers. Should these family members be treated with riluzole prior to the expression of the ALS phenotype? Since the potential protective role of riluzole and its safety in this situation is unverified, it needs to be made clear to individuals at risk that the role of this and other treatments is at present undetermined.

Table 4. Conditions misdiagnosed as ALS (From Rowland 1998)

ALS-mimic disorders

Cervical spondylotic myelopathy

Benign fasciculation

Primary lateral sclerosis and other forms of spastic paraparesis

Hexosaminidase deficiency

Spinobulbar muscular atrophy (Kennedy disease)

Monomelic spinal muscular atrophy (Hirayama syndrome)

Radiotherapy–myelopathy, neuronopathy, and neuropathy

Carpal tunnel syndrome

Inclusion body myositis

Hyperthyroidism

Polyglucosan body disease

Motor neuropathy neuropathy

Paraneoplastic motor neuron diseases

Monoclonal gammopathy

Lymphoproliferative disease

Breast cancer

Renal carcinoma

Multisystem disease: dementia, parkinsonism, cerebellar syndromes

AIDS, poliovirus, syphilis, Lyme disease, Creutzfeldt-Jakob disease

Table 5. Laboratory tests in the diagnosis of ALS (From Rowland, 1998)

Aim	Laboratory test
General health survey	Blood counts, erythrocyte sedimentation rate; urinalysis; serological test for syphilis; chest film
Confirm diagnosis ALS	EMG to show denervation in three limbs, tongue
Exclude unlikely causes	MRI of brain, cervical spinal cord Lyme antibodies Blood and urine for heavy metals
Exclude inclusion body myositis	Quantitative electromyography; muscle biopsy for rimmed vacuoles, amyloid stains
Exclude motor neuropathy	Peripheral nerve conduction studies in all patients, with or without upper motor neuron signs Motor nerve biopsy if no upper motor neuron signs
Exclude common carcinomas	Rectal and prostate examination Prostate specific antigen (men) Breast examination and mammogram (women) Bronchoscopy, biopsy any lesion found in chest film
Possible lymphoproliferative disease	Serum protein immunofixation electrophoresis; quantitative immunoglobulins; Anti-MAG, anti-GM1; anti-Hu Bone marrow biopsy if monoclonal protein found; other biopsy if lymphoma found CSF examination
Possible familial ALS, Kennedy syndrome	DNA analysis

1.5.4. Early diagnosis and diagnostic delay

With the introduction of riluzole and other therapeutical molecules in development or undergoing clinical trials, early diagnosis is increasingly important. A number of rationales supports the idea of early neuroprotective treatment (Cashman, 1999; Riviere et al., 1998). However, the strategy of early treatment can be debated since effects of current therapeutic regimens are moderate (Ludolph & Riepe, 1999). Nevertheless, experimental data obtained in transgenic animal models for ALS and Huntington's disease clearly show that early preclinical pathogenesis can be dramatically influenced by therapeutic interventions (Kong & Xu, 1998; Bates, 2000; Yamamoto et al., 2000). Moreover, early diagnosis applies not only to early treatment having more impact on the disease and hence on quality of life of the patient, but extends to providing greater scope for inclusion in clinical trials (Swash, 1998). In addition, the psychological interests of people with ALS and their families are best served by a diagnostic process which is efficient, direct, clear and unambiguous (Johnston et al., 1996).

Despite the introduction of riluzole therapy, the average time taken from symptom onset to ALS diagnosis seems to show no sign of improving. An initial incorrect diagnosis, refusal to consider the diagnosis when it was suggested by the informed patient, failure to consider a neurological cause for the patient's symptoms, and failure to make early referral to a neurologist may be among the major factors leading to delayed diagnosis (Househam & Swash, 2000). Consequently, there is a need of diagnostic markers able to screen phenotypically patients in early or preclinical disease stages. At present, there is only a single sensitive and specific marker for the diagnosis of ALS, the presence of mutations in the SOD1 gene. Otherwise, sensitive and specific diagnostic imaging, biochemical or genetic markers do not exist in ALS (Househam & Swash, 2000).

1.6. Measurements of disease progression

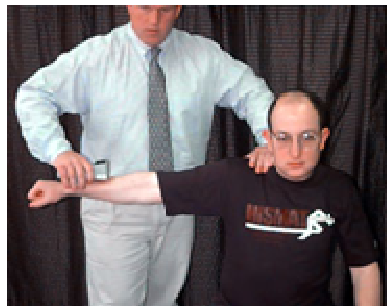
It is possible to determine motor neuron death and dysfunction in ALS directly. Quantitative techniques exist for the evaluation of lower motor neuron survival based on motor unit number estimation (Dantes & McComas, 1991). However, these techniques are cumbersome, painful, and are not readily applicable to more than one muscle per patient. Measures of upper motor neuron integrity are also bedeviled by a host of problems. Cortical magnetic stimulation has proved to be useful in the detection of impaired integrity of descending corticomotor neurons in ALS patients (Eisen et al., 1996; Claus et al., 1995), but with limited sensitivity. Imaging techniques has also been used, including MRI (Cheung et al., 1995; Mirowitz et al., 1989; Segawa, 1993; Udaka et al., 1992; Yagishita, 1995; Oba et al., 1993), PET (Kew et al., 1994) or single photon emission tomography (SPECT) (Abe et al., 1993). However, structural changes emerge late during the disease progression and are difficult to quantify, and both PET and SPECT are invasive techniques that require exposure to radioactive isotopes. The sensitivity and variability of these measures within a patient group make imaging techniques unsuitable for determination of disease progression in clinical trials.

Other indirect indicators of motor neuron degeneration have been used, including assays of muscle strength. Manual muscle testing (MMT) (Brooke et al., 1983; Medical Research Council, 1976) (Fig. 3) and maximal voluntary isometric contraction (MVIC) (Miller et al., 1999a; Andres et al., 1986; Andres et al., 1988; The National Isometric Muscle Strength (NIMS) Database Consortium, 1996) (Fig. 4) megascores have been used in recent clinical trials to assess treatment effect on strength. MMT may offer an advantage given the large number of muscles that can be sampled and the difference in cost, time and equipment required. Compared to MMT, MVIC is limited by the number of muscles sampled, the restrictions of time and patient fatigue associated with the technique, and the requirement of special equipment and technical expertise. Which has the best reproducibility and sensitivity is still debated (Aitkens et al., 1989; Andersen & Jakobsen, 1997; Hoagland et al.,

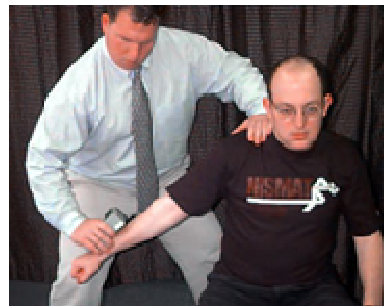
a



b



Start position



Final position

Copyright © 1996-2006 The Nicholas Institute of Sports Medicine and Athletic Trauma

Fig. 3. Manual muscle testing (MMT) of shoulder abductors.

MMT is a scored neurologic examination for assessment of muscle strength. The scale is numerical, with scores between 0 and 10, and is based on the examination of 34 muscles: neck flexors and extensors; shoulder abductors and external rotators; elbow flexors and extensors; wrist extensors and flexors; abductor pollicis brevis; flexor digiti minimi; hip flexors, extensors, and abductors; knee extensors and flexors; ankle dorsiflexors; plantar flexors; and extensor hallucis longus. Each muscle is scored from 0 to 5, with 0 representing paralysis and 5 normal strength and then converted to a 10-point scale. The final MMT score is the mean of the scores of all 34 muscles.

During MMT of shoulder abductors (shown in b), the test is begun by first stabilizing the patient with the arm abducted to approximately 90 degrees with the palm facing down, the subject's contralateral hand grabbing the side of the table to help stabilize. The examiner instructs the patient to resist as hard as possible and then gradually applies a downward force to depress the limb. The hand-held dynamometer (example shown in a) records the peak force during the movement.



From <http://www.uncg.edu/ess/anrl/equip.html>

Fig. 4. Maximal voluntary isometric contraction (MVIC).

MVIC may be measured quantitatively in multiple muscles, each one tested by a fixed load cell tensiometer interfaced with a computer for data acquisition and storage. MVIC may be measured in multiple muscle groups, including shoulder extension, elbow flexion, grip strength, hip flexion, knee extension, ankle dorsiflexion... Data are generally normalized, summed, and averaged to yield a megascore, which is then processed for data interpretation.

1997; Great Lakes ALS Study Group, 2003; Visser et al., 2003) but both techniques lack interobserver reproducibility, have to be performed by trained physical therapists and no consensus remains on which muscles and how many should be tested. Respiratory muscles activity is also measured by numerous tests. Forced vital capacity (total amount of air exhaled during a forced expiratory volume test, measured during spirometry) is commonly used but can remain normal despite substantial inspiratory muscle weakness. Maximal pressures measured at the mouth are useful for excluding weakness if they are normal but are difficult to interpret if abnormal. Invasive testing, such as measurement of transdiaphragmatic pressure, provides an accurate measure of inspiratory strength but is not readily available and is not practical for serial measures.

Disability and illness impact scales also provide indirect information about motor neuron degeneration. They include Norris ALS Scale (Norris et al., 1974), Appel ALS Scale (Appel et al., 1987), ALS Severity Scale (ALSSS) (Hillel et al., 1989), the sickness impact profile (Lai et al., 1997) and the ALS Functional Rating Scale (ALSFERS) (Table 6). ALSFERS is a dysfunction scale used in assessing the activities of daily living of patients with ALS. It was originally developed during the Regeneron CNTF phase I-II trials (The ALS CNTF treatment study (ACTS) phase I-II Study Group, 1996), based on the Unified Parkinson's Disease Rating Scale (Fahn & Elton, 1987) and the ALSSS, and revised by the brain-derived neurotrophic factor ALS Study Group Phase III (Cedarbaum et al., 1999). Although ALSFERS is a commonly accepted standard for monitoring disease progression used in clinical trials and in the treatment of ALS patients, much of what this scale is assessing are downstream functional effects of the disease, largely influenced by factors other than neuronal death, including individual motivation, family support, and financial resources. Moreover, its sensitivity, especially at the early stages of the disease, remains low.

Unfortunately, all such scales, including survival itself, are only related indirectly to the true biological lesion responsible for disease progression, namely the death and dysfunction of motor neurons. Moreover, none of these methods discriminate between upper and lower motor neuron involvement. Finally, all indirect

Table. 6. The amyotrophic lateral sclerosis functional rating scale (ALSFERS)

Function	Performance	Score
(1) Speech	Normal speech processes	4
	Detectable speech disturbance	3
	Intelligible with repeating	2
	Speech combined with nonvocal communication	1
	Loss of useful speech	0
(2) Salivation	Normal	4
	Slight but definite excess of saliva in mouth; may have nighttime drooling	3
	Moderately excessive saliva; may have minimal drooling	2
	Marked excess of saliva with some drooling	1
	Marked drooling; requires constant tissue or handkerchief	0
(3) Swallowing	Normal eating habits	4
	Early eating problems — occasional choking	3
	Dietary consistency changes	2
	Needs supplemental tube feeding	1
	NPO (exclusively parenteral or enteral feeding)	0
(4) Handwriting	Normal	4
	Slow or sloppy: all words are legible	3
	Not all words are legible	2
	Able to grip pen but unable to write	1
	Unable to grip pen	0
(5a) Cutting food and handling utensils (patients without gastrostomy)?	Normal	4
	Somewhat slow and clumsy, but no help needed	3
	Can cut most foods, although clumsy and slow; some help needed	2
	Food must be cut by someone, but can still feed slowly	1
	Needs to be fed	0
(5b) Cutting food and handling utensils (alternate scale for patients with gastrostomy)?	Normal	4
	Clumsy but able to perform all manipulations independently	3
	Some help needed with closures and fasteners	2
	Provides minimal assistance to caregiver	1
	Unable to perform any aspect of task	0
(6) Dressing and hygiene	Normal function	4
	Independent and complete self-care with effort or decreased efficiency	3
	Intermittent assistance or substitute methods	2
	Needs attendant for self-care	1
	Total dependence	0
(7) Turning in bed and adjusting bed clothes	Normal	4
	Somewhat slow and clumsy, but no help needed	3
	Can turn alone or adjust sheets, but with great difficulty	2
	Can initiate, but not turn or adjust sheets alone	1
	Helpless	0
(8) Walking	Normal	4
	Early ambulation difficulties	3
	Walks with assistance	2
	Nonambulatory functional movement	1
	No purposeful leg movement	0
(9) Climbing stairs	Normal	4
	Slow	3
	Mild unsteadiness or fatigue	2
	Needs assistance	1
	Cannot do	0
(10) Dyspnea (new)	None	4
	Occurs when walking	3
	Occurs with one or more of the following: eating, bathing, dressing (ADL)	2
	Occurs at rest, difficulty breathing when either sitting or lying	1
	Significant difficulty, considering using mechanical respiratory support	0
(11) Orthopnea (new)	None	4
	Some difficulty sleeping at night due to shortness of breath, does not routinely use more than two pillows	3
	Needs extra pillows in order to sleep (more than two)	2
	Can only sleep sitting up	1
	Unable to sleep	0
(12) Respiratory insufficiency (new)	None	4
	Intermittent use of BiPAP	3
	Continuous use of BiPAP during the night	2
	Continuous use of BiPAP during the night and day	1
	Invasive mechanical ventilation by intubation or tracheostomy	0

ALSFERS = SUM(score for all 12 measures)
--

ALSFERS is an instrument for evaluating the functional status of patients with ALS. It can be used to monitor functional change in a patient over time. The higher is the score, the more function is retained.

measurements of motor neuron integrity are subject to considerable variability, due to a variety of poorly controlled factors. From the perspective of clinical trials, adequate statistical power necessitates large numbers of patients observed over a long period of time, which makes proof of efficacy of a drug in ALS patients extremely expensive.

The development of biological markers to measure disease progression and their implementation in clinical trials may therefore be a necessary step to reduce both the required sample size and trial periods (Kaye, 2000). Biomarkers which have been advanced as potential surrogate markers include increased concentration of glutamate in CSF (Shaw et al., 1995; Spreux-Varoquaux et al., 2002) or in plasma (Iwasaki et al., 1992), abnormal concentration of kynurenic acid in CSF and serum (Ilzecka et al., 2003), increased level of prostaglandin E2 in CSF and serum (Ilzecka, 2003), elevated levels of creatine kinase in serum (Ilzecka & Stelmasiak, 2003a), decreased serum bilirubin concentration (Ilzecka & Stelmasiak, 2003b), increased serum levels of metalloproteinase-9 (Beuche et al., 2000) and transforming growth factor-1 (Houi et al., 2002). Elevated CSF or serum levels of markers of oxidative injury have been also proposed, including indices of DNA oxidative injury (Bogdanov et al., 2000), protein oxidation (Beal et al., 1997), and lipid peroxides (Smith et al., 1998; Simpson et al., 2004). Markers of apoptosis either in serum (Ilzecka et al., 2001; Sengun & Appel, 2003) or in blood (Poloni et al., 2000) have also been advanced. However, no relevant correlations with clinical features were found for these biomarkers. Therefore, there is a need to identify reliable and sensitive surrogate markers of the evolution of the disease.

2. Clinical management of ALS

2.1. Preventive therapy

Preventive therapy includes only relatives of SOD1-linked FALS patients. The availability of genetic testing for these families poses a dilemma : substances that are effective in the SOD1 transgenic mouse model (section 3.), such as creatine, riluzole, or vitamin E (Klivenyi et al., 1999; Gurney et al., 1996), may be potential preventive drugs for persons at risk for FALS but the uncertain benefits on human must be weighted against the burden of knowledge of their high-risk status as carriers of the mutation.

2.2. ALS-specific therapies

There is no satisfactory curative treatment for ALS. The antiglutamatergic agent riluzole has been estimated to prolong life by a few months in patients with ALS (Lacomblez et al., 1996). Around 10-15% of patients discontinue treatment because of side effects (asthenia, dizziness, gastrointestinal problems), and a few have to stop because of significant liver enzyme elevation. A Practice Advisory was issued summarizing the data on riluzole (Practice advisory on the treatment of amyotrophic lateral sclerosis with riluzole, 1997). A number of concerns about therapeutic effect persists: the lack of benefit observed in most secondary measures of efficacy, the quite modest prolongation of survival, and the relatively high cost of the drug.

Creatine is a nonprescription dietary supplement that has been shown to prolong life of SOD1 transgenic mice for twice as long as riluzole in the same model (Klivenyi et al., 1999). Two clinical trials of creatine in ALS have been completed without demonstration of significant improvements in overall survival or a composite measure of muscle strength (Groeneveld et al., 2003; Shefner et al., 2004).

Several attempts at finding a cure for ALS have failed so far, trophic factors being the most prominent example. However, there are ongoing trials of new

therapeutic agents, such as Ceftriaxone. There are also promising new drugs in the laboratory pipeline, such as vascular endothelial growth factor (VEGF) (Storkebaum et al., 2005; Azzouz et al., 2004)). In addition, recent findings guide researchers towards new therapies for the disease, such as gene therapy developments, stem cell research (Corti et al., 2004) or RNA silencing (Ralph et al., 2005).

2.3. Palliative care

Given the paucity of available curative options, palliative care is the mainstay of clinical management in ALS. It is a multidisciplinary approach that starts with the way the diagnosis is communicated and goes all the way to bereavement counseling for relatives after the patient's death. A first attempt at establishing evidence-based guidelines for clinical management of ALS has been published (Miller et al., 1999b) but many questions in this field have not yet been addressed.

3. Mutant SOD1 transgenic mice

The study of ALS has been hampered for a long time by the fact that no animal model was available. Studies of humans with ALS are complicated by the lack of genetic homogeneity or environmental uniformity. Moreover, possible early signs of disease that might appear prior to overt symptoms and could allow preemptive medical intervention are difficult to identify. A breakthrough in ALS was the discovery of a variety of mutations in the human SOD1 in about 20% of FALS cases (Rosen, 1993). This enabled the development of novel experimental models corresponding to transgenic mice expressing mutant forms of SOD1. To date, seven mouse models transgenic for mutant SOD1 have been generated (Wong et al., 1995; Bruijn et al., 1997; Ripps et al., 1995; Gurney et al., 1994; Friedlander et al., 1997; Dal Canto & Gurney, 1997; Dal Canto & Gurney, 1995) (Table 7). In mutant SOD1 mice, hind limb weakness appears as initial symptoms, followed by major symptoms such as progressive motor paralysis and neurogenic amyotrophy. These mice subsequently show disability of gait, eating and drinking, and die within some weeks. In our laboratory, we studied the SOD1 G86R mice (Ripps et al., 1995) (Fig. 5), that contain a transgene with the missense mutation Gly-86 → Arg, a mutation already observed in the corresponding amino acid residue of some patients with FALS (position 85, Fig. 2) (Rosen, 1993; Deng et al., 1993). Mutant SOD1 transgenic mice have provided many valuable insights into the pathogenesis of ALS, both by identifying early events in the neurodegenerative process and by attempting preemptive therapy.

Table 7. Transgenic mice expressing mutant SOD1 (Adapted from Shibata, 2001)

Mutation	Line - transgene copy number	SOD1 activity in CNS relative to control	Onset (days old)	Duration (days)	Reference
hG37R	42	14.5	120	ND	12
	9	9	150	ND	12
	106	7.2	180	ND	12
	29	7	210	ND	12
	148	1	240	7--14	13
hG85R	148	1	240	7--14	13
mG86R	M1	1	100	7	14, Fig. 5
hD90A	Hetero	Slightly increased	130	600	15
	Homo	Markedly increased	ND	ND	15
hG93A	G1H/+ - 25	13	90	<60	18
	G1L/+ - 18	11	130	<70	15,17
	G5/G5 - 10	7	290	>110	18
hG93R	ND	ND	230	10--13	16
hI113T	ND	Slightly increased	330	slow	X

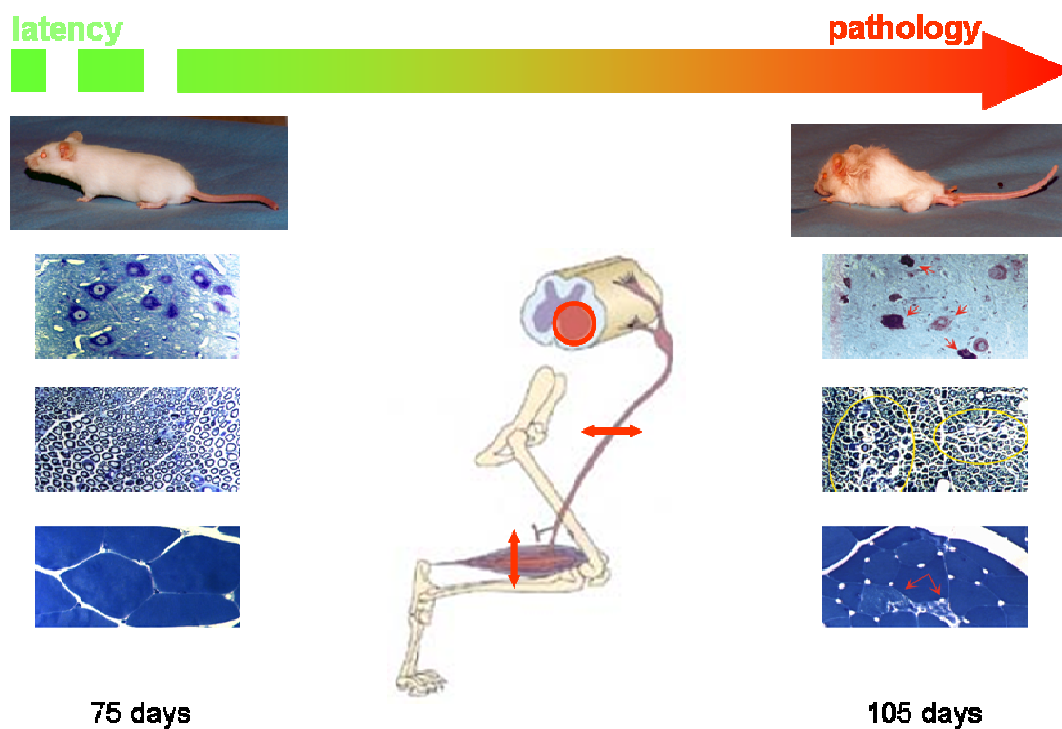


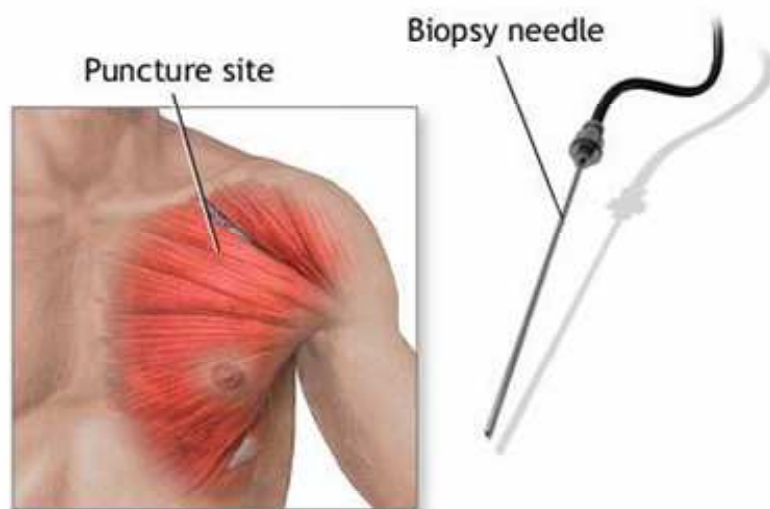
Fig. 5. The SOD1 G86R mouse is a model of ALS.

Histological analysis of the motor system on sections of lumbar spinal cord, sciatic nerve, and corresponding gastrocnemius muscle from 75- and 105-day-old SOD1 G86R mice. Sections were stained with toluidine blue. In 105-day-old mice tissues, note the decrease of motor neurons and the picnotic aspect of remaining perikarya (red arrows, first panel) in the lumbar spinal cord, the typical Wallerian degeneration of myelinated fibers in the sciatic nerve (yellow circles, second panel), and the muscle atrophy and degeneration (red arrows, third panel), compared to 75-day-old mice tissues.

4. Skeletal muscle in ALS

4.1. Skeletal muscle is a potential tool in ALS monitoring and diagnosis

Given the emergence of neuroprotective treatment strategies in ALS, there is a need to monitor ALS-modifying effects during clinical trials and to find ALS sensitive and specific markers for early diagnosis. Sections 1.5 and 1.6 of the introduction have shown that markers currently available are unsatisfactory, for lack either of sensitivity, specificity, reproducibility, safety, reliability, practicality, cheapness or quickness. Using skeletal muscle as a target to identify biological markers presents several advantages. Skeletal muscle is an easily accessible tissue by biopsy, needle biopsy is a little-invasive procedure (Fig. 6) and muscle biopsy is routinely performed for histopathological purposes. New technologies measuring gene expression such as quantitative polymerase chain reaction (QPCR) or microarrays needs little genetic material, may be rapidly applied to large number of subjects, and requires less and less specific equipment, technical expertise, cost and time. Moreover, these techniques provides quantitative, sensitive, reproducible and observer-independent data. Therefore, genetic regulations in ALS muscle that correlate with clinical parameters of the disease constitute potential markers of ALS progression. For diagnostic purposes, genes specifically regulated in ALS muscle constitute potential diagnostic markers of the disease. Finally, proteins coded by these regulated genes might be detected in biological fluids and constitute markers even cheaper and more suitable to perform serial examinations.



Copyright 2004 A.D.A.M., Inc. 

Fig. 6. Muscle biopsy procedure.

A muscle biopsy is a procedure involving the removal and examination of a piece of muscle tissue. It can usually be obtained while you are awake and the area being biopsied is numbed by local anesthesia. No fasting or other special preparation is usually necessary. In a needle biopsy procedure, a needle is inserted into the muscle and a small "plug" of tissue remains in the needle when it is removed from the muscle. During the biopsy, there is usually minimal or no discomfort. The anesthetic may burn or sting when injected before the area becomes numb. After the anesthetic wears off, the area may be sore for about a week.

4.2. Potential involvement of skeletal muscle in ALS etiology

There is a general acceptance that ALS is a motorneuron disease. However, current theories are shifting from an exclusive motor neuron pathology to a focus on the interaction between motor neurons and other cell types. Indeed, targeted expression of mutant SOD1 either in motor neurons (Pramatarova et al., 2001; Lino et al., 2002) or in astrocytes (Gong et al., 2000) does not lead to an ALS-like phenotype, suggesting that these cell types may not be primarily involved in ALS pathogenesis. Moreover, SOD1 mutant neurons survive longer when surrounded by an environment having a high proportion of wild-type nonneuronal cells, supporting the view that the cellular background of motor neurons is a determining factor in the degeneration process caused by SOD1 mutations (Clement et al., 2003). Damaged nonneuronal cells and motor neurons, therefore, could act in concert to provoke the disease. Many studies focused on the role of astrocytes in the motor neuron loss in ALS (Barbeito et al., 2004), most likely due to their close interactions, astrocytes providing structural, metabolic and trophic support to neurons, and actively participating in modulating neuronal excitability and neurotransmission. However, little is known about the influence exerted on motor neurons by other cells types, including skeletal muscle cells.

Examination of muscle biopsy samples from ALS patients by electron microscopy revealed ultrastructural abnormalities of muscle mitochondria (Chung & Suh, 2002). These abnormal mitochondria are associated with mitochondrial dysfunctions in skeletal muscle of ALS patients (Siciliano et al., 2001; Vielhaber et al., 1999; Vielhaber et al., 2000; Wiedemann et al., 1998). These findings, together with many different metabolic alterations observed in the disease, have contributed to the emergence of the quite recent metabolic hypothesis in ALS pathogenesis [(Gonzalez de Aguilar et al., 2005) for review]. In this paradigm, failure in skeletal muscle, a metabolically important tissue, may constitute an additional driven force to increase motor neuron vulnerability. Interestingly, the hypothesis making the skeletal muscle an active player for initiating the development of ALS takes account of an essential characteristic of the disease, which is isolated involvement of the motor system.

Other data support primitive muscle involvement in ALS. Studies in G93A mice revealed an increase in SOD activity during progression of disease in muscles, but not in nervous tissue, associated with changes in mitochondria respiratory properties in oxidative muscle fibers (Leclerc et al., 2001). These findings suggest that oxidative stress due to SOD1 mutations could alter energy metabolism in FALS mice, thereby affecting primarily oxidative muscle of the limbs, independently of motor neuron loss. Besides, our recent results on SOD1 mice are strongly indicative of the early and long-lasting activation of a series of molecular effectors thought to act coordinately in preventing the increased oxidative stress in ALS muscle [Article 4]. Another study in G93A mice revealed by MRI a decrease in hindlimb muscle volume, before other overt pathology appeared. In line with these findings, many studies in G93A mice have demonstrated dysfunction of the neuromuscular junction long before the loss of motor neurons are reported. Whereas significant loss of motor neurons is not detected before 80-90 days in G93A mice (Chiu et al., 1995; Kennel et al., 1996), selective loss of fast-firing neuromuscular synapses as early as day 50 (Frey et al., 2000), progressive loss of motor unit numbers beginning at day 40 (Kennel et al., 1996), and 40% of end-plates denervation at day 47 (Fischer et al., 2004) have been reported. Moreover, autopsy of a patient with SALS demonstrated denervation and reinnervation changes in muscle but normal appearing motor neurons (Fischer et al., 2004). Altogether, these observations imply that ALS is actually a "dying-back" motor neuropathy where distal axonal degeneration occurs early during the disease, before neuronal degeneration and onset of motor symptoms, at least in the widely studied G93A mice and in a human case.

Skeletal muscle may be an important actor in this "dying-back" process, by controlling the terminal sprouting. Thus, local retrograde signaling does play a role in regulating nerve growth at the synapse, notably during development of the nervous system with cell adhesion molecules like N-CAM and N-cadherin or with soluble trophic factors like brain-derived neurotrophic factor and ciliary neurotrophic factor. Not only developing but adult muscle is a source of signals that influence neuron survival, axonal growth, and maintenance of synaptic connections (Funakoshi et al., 1995). In accordance with this, overexpression of glial cell line-derived neurotrophic factor (GDNF) by muscle greatly increased the number of motor axons innervating neuromuscular junctions in neonatal mice (Nguyen et al., 1998). Most interestingly, muscle-restricted expression of a localized insulin like growth factor-1 (IGF-1) isoform

in G93A mice stabilized neuromuscular junctions, reduced inflammation in the spinal cord, and enhanced motor neuronal survival, delaying the onset and progression of the disease (Dobrowolny et al., 2005). Similarly, viral muscle delivery of GDNF (Acsadi et al., 2002; Wang et al., 2002b; Mohajeri et al., 1999; Manabe et al., 2002; Lu et al., 2003) or IGF-1 (Kaspar et al., 2003) can increase survival in G93A mice. A possible origin for a deleterious retrograde signaling could be metabolic alterations in skeletal muscle, which might contribute to an "undernourishment" of the most distal region of the axon.

Objectives and Organization of the Results

ALS is emerging as a “multi-system” disease in which the alteration in both muscle, motor neuron and glia cells may act synergistically to induce the disease. Although skeletal muscle is quite an untested component in the motor neurodegenerative process in ALS, findings described in the introduction establish skeletal muscle as a promising primary target in ALS research. Indeed, skeletal muscle is of greatest interest both as a clinical tool in monitoring and diagnosing ALS patients, and as a primary actor in ALS pathogenesis. Therefore, the aim of the present PhD work was to explore gene expression modifications in ALS skeletal muscle in order to identify molecular markers of the disease. Two independent approaches were conducted in parallel using muscle as a starting point. First, our gene expression studies targeted two gene families, focusing on two proteins particularly relevant as molecular markers of ALS, named Nogo-A and UCP3, described respectively in chapter 1 and chapter 2 of the results. Second, we extensively explored the transcriptome of ALS muscle using a DNA microarray approach described in chapter 3. This systematic strategy enabled us to establish a molecular identity card of skeletal muscle affected by ALS and identify two groups of genes of particular interest from a clinical point of view.

Results and Discussion

Chapter 1

Nogo-A is a potential marker of ALS

1. Nogo-A is a potential marker of ALS

1.1. Reticulon family

Reticulons are a family of highly conserved proteins (Oertle et al., 2003b), which are ubiquitously expressed in vertebrates and have also been described in *Drosophila melanogaster*, *Schizosaccharomyces pombe* (Godoy et al., 1996) and *Caenorhabditis elegans* (Iwahashi et al., 2002). Initially described as specific for neural tissue (Wieczorek & Hughes, 1991; Kools et al., 1994) and markers for some types of lung cancer (Roebroek et al., 1993), these proteins were called neuroendocrine-specific proteins, owing to its neuroendocrine-specific expression. Due to their preferred intracellular localization in the endoplasmic reticulum (ER), they were renamed reticulons (RTNs) (van de Velde et al., 1994a).

In mammals, four genes (*rtn1*, *rtn2*, *rtn3* and *nogo/rtn4*) lead to the expression of multiple alternative transcripts (Fig. 7). They all share a homologous carboxyl terminus composed of ~200 amino acids and called the reticulon homology domain (RHD). RHD features two large hydrophobic regions, potentially corresponding to two transmembrane domains, and a ~66 amino acids loop in between. RTN1, 2 and 4 are expressed as multiple N-terminal isoforms that are generated by differential promoter usage or alternative splicing (Oertle et al., 2003a). Since RTN3 was the only RTN known to exist in only one isoform, bearing a very short N-terminal domain and the RHD (Hamada et al., 2002; Moreira et al., 1999), we analysed databases for potential new RTN3 isoforms [Article 1]. We described a revised genomic structure of mouse and human gene *rtn3*, which contain nine exons that may encode up to eight putative gene products. Only five of these variants were present at the mRNA and protein levels.

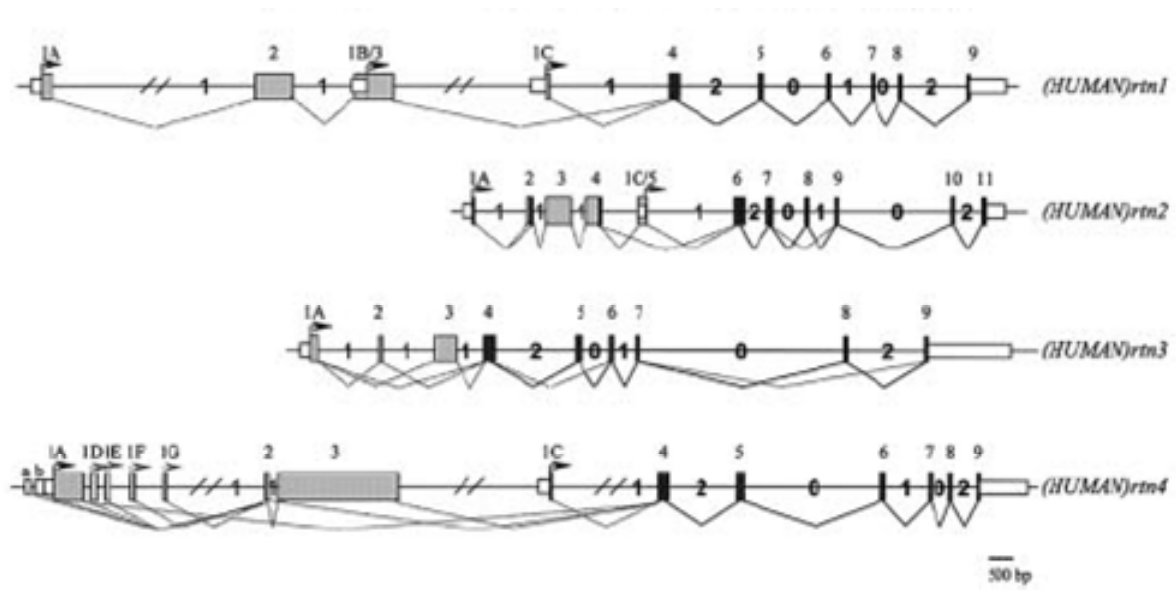


Fig. 7. Exon-intron structure of the four mammalian RTN genes (From Oertle, 2003).

Exons coding for the RHD are indicated in black, in gray those coding for the amino termini, and in white boxes the UTRs. Arrows indicate the translation start sites. Numbers designate intron phasing. Exon sizes are matched according to the indicated line.

1.2. Functions of mammalian RTNs

At present, little is known about the functions of RTNs [(Oertle & Schwab, 2003) for review]. In the ER, RTN1-A forms aggregates with RTN1-B (Senden et al., 1994a), but not with RTN1-C (Senden et al., 1996). Such a complex is thought to work as a pore-like structure but evidence supporting this notion is still lacking. Similarly, Nogo-A could also form a membrane channel or transporter by interacting with its isoform partners Nogo-B and Nogo-C (Dodd et al., 2005). RTNs do not only associate with the ER but also with other cellular constituents, including the plasma membrane and the Golgi apparatus (Chen et al., 2000; GrandPre et al., 2000). Thus, RTN1-C and RTN3 have been proposed to participate in the trafficking of proteins between the ER and the other membrane compartments (Steiner et al., 2004; Wakana et al., 2005).

The presence of multiple putative SH3-domain-binding sites in the N-terminal region of Nogo (Oertle et al., 2003a) has suggested the occurrence of protein-protein interactions. Such interactions have been already reported, including RTN2 interaction with the intermediate filaments in the Z-bands of muscle sarcomeres (Geisler et al., 1999), Nogo interaction in nervous tissue with ubiquinol-cytochrome c reductase core protein II and with NIMP (Nogo-interacting mitochondrial protein, a new mitochondrial protein) (Hu et al., 2002), as well as with alpha-tubulin and myelin basic protein (Taketomi et al., 2002), and RTN1-A and RTN1-B interaction with the AP-2 adaptator complex involved in endocytosis (Iwahashi & Hamada, 2003). These interactions may account for novel functions of RTNs.

Several studies reported the implication of RTNs in apoptosis. RTN1-C and Nogo-B interact with the anti-apoptotic proteins Bcl-xL and Bcl-2 sequestering them from mitochondria to the ER and hence preventing their anti-apoptotic actions (Tagami et al., 2000). RTN1-C also interacts with the pro-apoptotic enzyme glucosylceramide synthase to modulate its catalytic activity (Di Sano et al., 2003). Upon cell death of cerebellar granule neurons, RTN3-A1, RTN3-A2 and RTN3-A4 expression is repressed, probably through an apoptotic-dependent process [Article 1]. Concerning Nogo-B, its pro-apoptotic function is still debated (Li et al., 2001; Qi et al., 2003; Tambe et al., 2004; Oertle et al., 2003c). In addition to apoptosis, growing

data endow RTNs with new roles in many different tissues, including vascular remodeling in endothelial and smooth muscle cells (Acevedo et al., 2004), response to hydrostatic pressure in chondrocytic cells (Sironen et al., 2004) and amyloid-beta metabolism modulation in human brain (He et al., 2004).

Most of the existing literature on RTNs deals with the role of Nogo as a potent inhibitor of axonal growth and repair. Nogo-A, the longest Nogo isoform, was initially characterized as a myelin fraction protein recognized by a monoclonal antibody able to neutralize its growth-inhibitory activity (Caroni & Schwab, 1988). This growth cone collapse activity is presently known to occur mainly through the interaction of the Nogo-66 loop with a receptor complex, formed by a glycosyl phosphatidyl inositol-anchored membrane receptor called NgR (Fournier et al., 2001), the low-affinity nerve growth factor receptor p75NTR (Wang et al., 2002a), and a nervous system-specific transmembrane protein named LINGO-1 (Mi et al., 2004). In addition to this receptor complex, TROY, an orphan tumor necrosis factor receptor family member widely expressed in the nervous system, may also interact with NgR and LINGO-1 to mediate the inhibitory action of Nogo-A (Park et al., 2005; Shao et al., 2005). Neurite outgrowth inhibition is also supported by two additional Nogo-A-specific active fragments in the N-terminal region, named NiR- Δ 2 and NiG- Δ 20, which also prevent fibroblast spreading (Oertle et al., 2003d). Although acting via different neuronal binding sites, the actions of both Nogo-66 and NiG- Δ 20 converge at the Rho-GTPases RhoA and Rac1 to inhibit neurite outgrowth (Schweigreiter et al., 2004). Most of Nogo-A is retained in the ER but it is also present at the cell membrane of oligodendrocytes (Nie et al., 2003). Therefore, it is assumed that the exposure of Nogo-A and NgR at the interface between neurons and oligodendrocytes is in part responsible for the failure of CNS regeneration. Although regeneration abilities of Nogo knockout mice are still debated (Simonen et al., 2003; Kim et al., 2003; Zheng et al., 2003), many studies, reporting both findings on Nogo/Nogo receptor distribution (Josephson et al., 2001; Huber et al., 2002; Buss et al., 2004; Al Halabiah et al., 2005), and functional findings (Emerick et al., 2003; Mingorance et al., 2004; Lee et al., 2004; Kim et al., 2004; Li et al., 2004), support Nogo as an important actor in the modulation of axonal pathfinding during development and in the adaptive response of the adult CNS to axonal injury.

1.3. RTNs as disease markers

1.3.1. RTNs in cancer

RTNs were initially described as markers of neural and neuroendocrine differentiation in normal and malignant cells. Thus, RTN1-A and RTN1-C are expressed in most of the small-cell lung carcinoma (SCLC) and carcinoids, whereas they are absent in non-SCLC tumors (except for non-SCLCs exhibiting neuroendocrine features that also show RTN1-A expression) (van de Velde et al., 1994b; Senden et al., 1997b; Senden et al., 1994b). Serum autoantibodies to Nogo-A are a frequent finding in pilocytic astrocytoma patients (Michel et al., 1999). Similarly, overexpression of RTN3 was reported in astrocytoma tumor cells, as compared to non-cancerous brain tissue. Therefore, RTN antibodies might serve as novel markers for these cancerous diseases.

1.3.2. RTNs in neurological diseases

Despite the important role that RTNs seem to play in the nervous system, little is known about the implication of these proteins in neurological diseases. Reductions of RTN1-C levels have been reported in cortex from Down syndrome and Alzheimer's disease (AD) patients (Kim et al., 2000). RTN family members have been shown to bind β -amyloid converting enzyme 1 (BACE-1), the type I transmembrane aspartyl protease reported to be the β -secretase involved in AD. *In vitro* changes in RTN3 expression markedly modulate BACE1 protease activity, suggesting that changes in RTN expression in the human brain are likely to affect cellular amyloid- β and the formation of amyloid plaques involved in AD pathogenesis (He et al., 2004).

Serum autoantibodies to Nogo-A are a frequent finding in MS, acute inflammatory and non-inflammatory neurological diseases (Reindl et al., 2003). High levels of Nogo-A mRNA were detected in frontal cerebral cortices from patients with schizophrenia (Novak et al., 2002). Increased expression of Nogo-A was also observed in hippocampal neurons of patients with temporal lobe epilepsy (Bandtlow et al., 2004) and in rat hippocampus in a kainic acid model of this disease (Meier et

al., 2003). According to these observations and those reporting the neuroprotective effect of Nogo-A vaccination on the lesioned spinal cord (Hauben et al., 2001; GrandPre et al., 2002), it was proposed that vaccination against Nogo-A was an interesting therapeutical approach for neurological diseases (Merkler et al., 2003). In this regard, targeting Nogo-A by active immunization suppresses clinical and histological signs associated with experimental autoimmune encephalomyelitis, a model of MS (Karnezis et al., 2004). However, the type of recruited immune response may negatively influence the effects of this kind of therapeutical approach (Fontoura et al., 2004).

1.3.3. RTNs in ALS

We showed that G86R mice and patients with sporadic ALS exhibit a specific pattern of Nogo expression in skeletal muscles [Article 2]. High levels of Nogo-A protein were found in ALS patients, whereas Nogo-A had been barely detectable in muscles of normal subjects, of primary muscle disease and of polyneuropathy patients. Moreover, our team has recently demonstrated that Nogo-A immunoreactivity in skeletal muscle correlates with the severity of motor impairment as assessed by the ALSFRS, and with the degree of muscle fibers atrophy in patients with sporadic ALS (Jokic et al., 2005). These observations suggest that Nogo-A expression in muscle could be used as a marker of ALS functional disability and in monitoring the disease-modifying effects during therapeutic trials.

1.3.4. RTNs are linked to differentiation processes

Many alterations in RTNs expression have been linked to differentiation, both in nervous and muscle tissues. Indeed, RTN1 proteins are considered to combine a high sensitivity with a high specificity for detection of neuroendocrine differentiations in lung cancer (Senden et al., 1997a). Moreover, RTN1-C expression is strongly correlated with the degree of neuronal differentiation in human neuroblastoma cell lines (Hens et al., 1998), and is also up-regulated during aging of primary cultures of cerebral cortical neurons (Li et al., 2002) and down-regulated after maturation of the

optic nerve (Kumamaru et al., 2004). We showed that RTN3 and Nogo proteins increased during cerebellar development and during *in vitro* development of cerebellar granule neurons, and decreased during *in vitro* cell death [Article 1]. Denervation, which is known to be associated with reactivation of myogenic or de-differentiation events that come into play to compensate muscle atrophy, cause decreased Nogo-C and increased Nogo-A expression in skeletal muscle (Magnusson et al., 2003). We recently observed up-regulation of Nogo-C and RTN3-C in skeletal muscle following birth and during *in vitro* differentiation of primary myoblasts [Fergani et al., in revision]. Therefore, some of the RTN expression alterations observed in pathological cases might reflect the differentiation or de-differentiation component of the disease associated with the degenerative changes.

Tissue specificity and regulation of the N-terminal diversity of reticulon 3

Di Scala F, Dupuis L, Gaiddon C, De Tapia M, Jokic N,
Gonzalez de Aguilar JL, Raul JS, Ludes B, Loeffler JP

Nogo provides a molecular marker for diagnosis of amyotrophic lateral sclerosis

Dupuis L, Gonzalez de Aguilar JL, Di Scala F, Rene F, de Tapia M,
Pradat PF, Lacomblez L, Seihlan D, Prinjha R, Walsh FS, Meininger V,
Loeffler JP

Chapter 2

Uncoupling protein 3 is a potential marker of ALS

2. Uncoupling protein 3 is a potential marker of ALS

2.1. Uncoupling protein family

Uncoupling proteins (UCPs) belong to the superfamily of mitochondrial anion-carrier proteins. Up to five different uncoupling protein types (UCP1, UCP2, UCP3, UCP4 and UCP5/BMCP1) were found in mitochondria of mammals and plants, and have also been described in fishes, fungi and protozoa. The archetypal uncoupling protein, UCP1, formerly known as thermogenin, is mainly found in brown adipose tissue and mediates proton leak in brown adipocytes to produce heat in response to cold exposure. UCP2 and UCP3 have high sequence identity with UCP1 (Borecky et al., 2001) and were isolated as homologues of UCP1 (Fleury et al., 1997; Gimeno et al., 1997; Boss et al., 1997; Vidal-Puig et al., 1997; Gong et al., 1997). Originally it was thought that these additional UCPs functioned in a thermoregulatory manner similar to UCP1, but their uncoupling activity is still debated and their physiological function remains still unknown (Goglia & Skulachev, 2003; Jaburek et al., 2004; Boss et al., 2000).

2.2. UCP2 and UCP3 expression is increased in ALS muscle

Studies on UCP2 (Arsenijevic et al., 2000) and UCP3 knockout mice (Gong et al., 1997; Vidal-Puig et al., 1997) showed that these proteins might be involved in the regulation of mitochondrial respiration and that this function might deal with the resistance to oxidative stress. Since growing evidence points to mitochondrial dysfunction as playing a crucial role in ALS pathogenesis [(Gonzalez de Aguilar et al., 2005) for review] and that damage by reactive oxygen species (ROS) is involved in ALS, we monitored UCPs expression pattern in ALS [Article 3]. We showed that UCP2 and UCP3 are increased in ALS skeletal muscle, both in G86R mice and in human ALS biospsies. Since UCP3 induction was not observed in muscle biopsies of

polyneuropathies and primary muscle diseases patients, UCP3 is a potential marker of ALS.

2.3. UCP3 is a potential modulator of reactive oxygen species metabolism in ALS

ROS regroup molecules such as superoxide anion, hydroxyl radical and hydrogen peroxide, possess strong oxidative capacity and are normal by-products of mitochondrial respiration. However, ROS produced in excess can mediate cellular damage, which is known to contribute to ALS pathogenesis [(Simpson et al., 2003) and (Liochev & Fridovich, 2003) for review]. The potential role for UCP3 in modulating ROS formation in muscle is supported by many studies. For instance, mice lacking UCP3 exhibited an increase in ROS level in their muscles (Vidal-Puig et al., 2000) and increased UCP3 expression associated with hyperoxia-mediated oxidative stress was recently reported, both in mouse muscle and in C2C12 myotubes (Flandin et al., 2005). Moreover, a role for UCP3 in the regulation of ROS production also fits with the finding that UCP3 expression decreases with aging in mitochondria isolated from rat skeletal muscle, because aging has been associated with increased ROS production (Kerner et al., 2001). Furthermore, UCP3 induction in human skeletal muscle after acute exercise (Pilegaard et al., 2000) could serve to prevent the well-known increase of ROS production during exercise. In the light of these findings, we hypothesize that UCP3 induction we observed in ALS muscle might participate in a counter-regulatory mechanism to lower the production of ROS due to oxidative stress, through a yet unknown mechanism but possibly in a manner that is independent of mitochondrial uncoupling (Maclellan et al., 2005). Therefore, UCP3 induction might be among the numerous intracellular antioxidant defences already observed in ALS muscle [Article 4]. It is also worth emphasizing that ROS at their turn might regulate UCP3 activity (Echtay et al., 2002), which could contribute to the antioxidant response of ALS muscle.

2.4. UCP3 is a potential mediator of hypermetabolism in ALS

Growing evidence points to metabolic perturbations as playing an important role in ALS. Creatine, an intracellular energy shuttle between mitochondria and sites of energy consumption, extends the life expectancy of G93A mice (Klivenyi et al., 1999). Our team has shown important variations in a number of metabolic indicators in G86R mice, which in all shows a metabolic deficit (Dupuis et al., 2004). These alterations were accompanied early in the asymptomatic phase of the disease by reduced adipose tissue accumulation, increased energy expenditure, and concomitant skeletal muscle hypermetabolism. Compensating this energetic imbalance with a highly energetic diet extended mean survival by 20%, a significant effect when compared to other beneficial treatments (Dugan et al., 1997; Gurney et al., 1996; Zhu et al., 2002). In addition, at least a subset of ALS patients shows a characteristic hypermetabolism phenotype (Kasarskis et al., 1996; Desport et al., 2001), reminiscent of that observed in G86R mice. It is also worth emphasizing that the nutritional status is a prognostic factor for survival in ALS (Desport et al., 1999), and that an appropriate, individualized nutritional management of patients may constitute a primary symptomatic treatment for the disease (Cameron & Rosenfeld, 2002).

Many studies provided evidence for the *in vitro* (Gong et al., 1997; Vidal-Puig et al., 2000; Clapham et al., 2000) and *in vivo* (Cline et al., 2001) uncoupling activity of UCP3. Moreover, this uncoupling activity is already known to mediate hypermetabolism responsible of hyperphagy and leanness of muscles from transgenic mice that overexpress UCP3 (Clapham et al., 2000). In ALS, existence of mitochondrial uncoupling is suggested by decreased respiratory control ratio of isolated mitochondria and decreased levels of ATP in G86R muscle tissue [Article 3], and other indirect evidence (Carri et al., 1997). Therefore, UCP3 induction may participate to the hypermetabolism observed in ALS through mitochondrial uncoupling. However, the mechanism by which UCP3 uncouples mitochondria is still under debate (Klingenberg et al., 1999; Jezek et al., 1998) and further studies are needed both to validate the link between muscular induction of UCP3, mitochondrial uncoupling and hypermetabolism, and to determine the role of hypermetabolism in ALS pathogenesis.

**Up-regulation of mitochondrial uncoupling protein 3
reveals an early muscular metabolic defect
in amyotrophic lateral sclerosis**

Dupuis L, Di Scala F, Rene F, de Tapia M,
Oudart H, Pradat PF, Meininger V, Loeffler JP

Early activation of antioxidant mechanisms in muscle of mutant Cu/Zn-superoxide dismutase-linked amyotrophic lateral sclerosis mice

Jokic N, Di Scala F, Dupuis L, Rene F,
Muller A, Gonzalez De Aguilar JL, Loeffler JP

Chapter 3

Expression profiling of skeletal muscle in patients affected by ALS

3. Expression profiling of skeletal muscle in patients affected by ALS

3.1. The microarray approach

Microarrays, a recently developed technology, have known a tremendous progress and have become a revolutionary platform for the study of gene expression, as can be seen from the exponential increase in the number of research articles using microarray-based technology (Fig. 8). Microarray-based investigations, combined with advanced bio-informatics, hold tremendous potential both in the study of biological processes in disease and in molecular diagnosis. Notable examples include the identification of genes that classify subtypes of cutaneous melanoma (Bittner et al., 2000); genes that predict survival for diffuse large B-cell lymphoma (Shipp et al., 2002; Alizadeh et al., 2000), breast cancer (Perou et al., 2000; Sorlie et al., 2001; van 't Veer et al., 2002) and lung adenocarcinoma (Beer et al., 2002); and genes that open new therapeutic targets for MS (Lock et al., 2002).

3.2. The technology of microarray

A glossary of common terms in microarray literature used in this section is provided (see term*). Microarrays*, also known as biochips, DNA chips and gene chips, may be described as an ordered array of microscopic elements on a planar surface that allows the specific binding of genes or gene products (Schena, 2002). An effective microarray requires that all analytical elements have their own unique address and are equally sized and uniformly configured. This allows accurate identification during analysis and subsequent ease in examination and quantification. Various types of microarrays have been produced based on their target material,

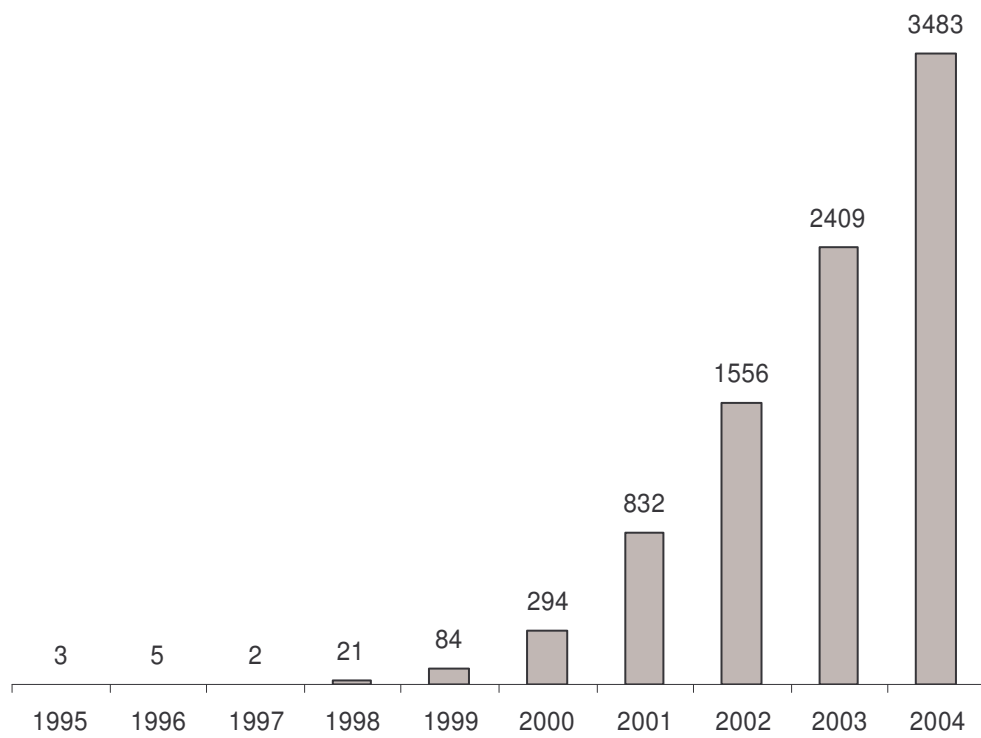


Fig. 8. Number of microarray based publications since 1995.
Analysis performed on PubMed database.

which can be cDNA, mRNA, protein, small molecules, tissues or any other material that allows quantitative gene analysis. We used high-density oligonucleotide microarrays (Lockhart et al., 1996), manufactured by Affymetrix and commercially named GeneChips, which will be the common name used here. Two nomenclatures exist regarding the definition of “target”^{*} and “probe”^{*}. Certain authors use the term “target” to describe the molecule that is attached to a microarray substrate and reacts with the free “probe” which is in solution. Here, “target” has been defined as the free nucleic acid and “probe” as the nucleic acid attached to the microarray substrate.

The GeneChip used in our experiments was the GeneChip Human Genome U133 Set (HG-U133A and HG-U133B) (Table 7 for critical specifications). As previously described (Lipshutz et al., 1999), HG-U133 arrays are prepared by depositing 25-nucleotide molecules on a quartz surface and two types of probes are used, called Perfect Match^{*} (PM) and Mismatch^{*} (MM) probes. The PM probes match a target sequence exactly, while its partner probe, the MM probe, differs from the PM probe by a single base in the middle of the sequence. PM and MM probes are together known as a probe pair, and 11 probe pairs^{*} constitutes a probe set^{*}, which is used to investigate different parts of the sequence of the same gene (Fig. 9). The expression measure of a determined gene is the average of the information obtained from each of the probes in a probeset.

The technology of GeneChips is characterized by very interesting capabilities:

i) Sensitivity: arrays are produced with hundreds of thousands of different probes packed at an extremely high density, allowing genome-wide data using small sample volumes

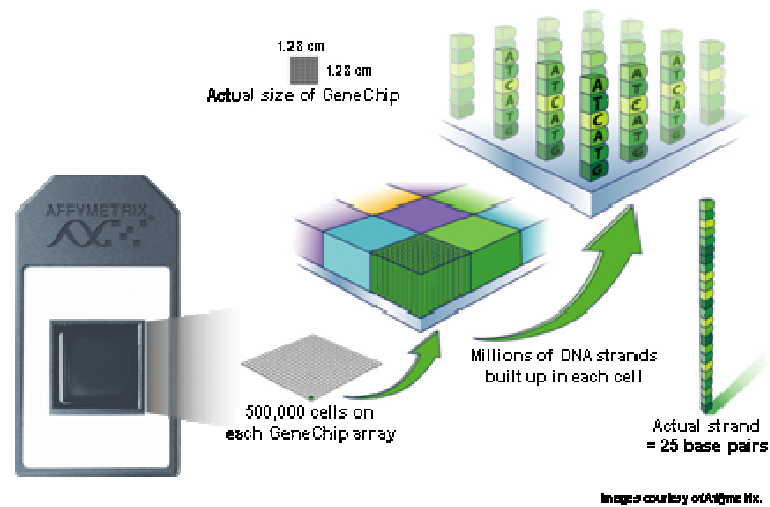
ii) Specificity: the PM/MM probe pairs allow the quantitation and subtraction of signals caused by non-specific cross hybridization, finally indicating specific target abundance

iii) Reproducibility: the design and manufacture of these arrays are highly stereotyped and consistent, providing a high degree of reproducibility between experiments and enabling independent examination of the same gene in the experiment.

Table 7. Critical specifications of GeneChip Human Genome U133 Set (From Affymetrix website)

Number of array in Set	2
Number of probesets	>45 000
Number of transcripts	~39 000
Number of genes	~33 000
Number of features	>1 000 000
Feature size	18 μ m
Oligonucleotide probe length	25 mers
Probe pairs/sequence	11
Housekeeping/control genes	GAPDH, beta-actin, ISGF-3
Databases used in the design of the array	2,7 million input sequences selected from GenBank, dbEST, RefSeq

a



b

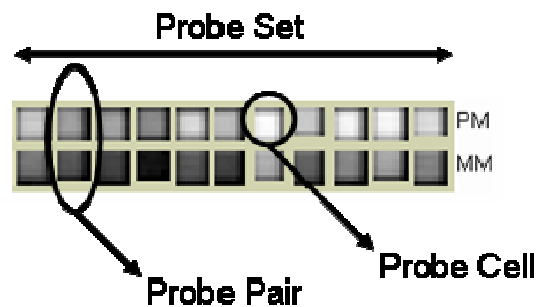


Fig. 9. GeneChips content.

a. Oligonucleotides of 25 base pairs are synthesized onto a quartz support of 1.28x1.28 cm. Several millions identical 25mers fit into a feature or a probe cell of about 18 μm .

b. The organization of an array is as follows. One transcript is quantified by one probe set and a single array may contain probe sets for more than 40,000 human genes and ESTs. One probe set is composed by 11 probe pairs. One probe pair is composed by a perfect match (PM) probe cell and a mismatch (MM) probe cell. A PM probe is an oligonucleotide that is complementary to a transcript and a MM probe is an oligonucleotide with the corresponding sequence containing a point mutation. Oligonucleotide probes are chosen based on uniqueness criteria and composition design rules. The use of the differences averaged across a set of probes greatly reduces the contribution of background and cross-hybridization.

3.3. Gene expression experiment

We analysed deltoid muscle biopsies obtained from ten ALS patients (from Prof. Vincent Meininger's Service - Hôpital Pitié-Salpêtrière) and from ten controls without nervous system pathology (from Banque de Tissus pour la Recherche - Association Française contre les Myopathies). All ALS patients were in early stages of the disease, except for two patients more advanced in the pathology (see Table 8 for clinical features of ALS patients and control subjects).

One of the major concerns in microarray profiling studies of clinical samples is the effect of tissue sampling and RNA extraction on data. To keep variations from technical sources at minimum, muscle biopsies were collected by the same surgeon (one for ALS patients, another one for control subjects), snap-frozen within 1 minute following surgical resection and stored until RNA extraction in liquid nitrogen. In addition, special attention was taken at each step of the target preparation to protect RNA against high temperature, temperature changes and RNases. For fibrous tissues such as skeletal muscle, the most difficult step in the isolation process can be complete disruption of all the cells when preparing tissue homogenates. Due to low cell density and the polynucleate nature of muscle tissue, biopsies are indeed difficult to homogenize completely and can result in degraded RNA and very low yield. To overcome these difficulties, we extracted total RNA using the FastPrep® system (Fig. 10 for the overall RNA extraction procedure). Total RNA and cRNA target quality were assessed using the Agilent Bioanalyzer system as described in Fig. 11. Purified total RNA served as template to synthesize the biotin-labeled cRNA target, and GeneChips were scanned after target hybridization and automated chip washing/staining (Fig. 12). Images obtained from the chips were computed as described in the next section.

Table 8. Clinical features of the ALS patients and control subjects analysed by GeneChips

ALS patients	age (years)	gender	disease duration (days)	ALSFRS score	right arm abduction score	control subjects	age (years)	gender
1	52	M	491	43	5	1	50	M
2	36	M	371	40	4	2	55	F
3	54	M	194	41	5	3	65	F
4	48	M	425	28	1	4	57	M
5	50	M	576	42	5	5	45	F
6	62	M	401	43	5	6	18	M
7	60	M	400	42	5	7	48	F
8	49	M	491	28	2	8	60	F
9	58	M	491	45	4	9	29	M
10	54	M	221	43	4	10	25	M

Most of ALS patients were in early stages of the disease, as indicated by high ALSFRS scores (between 40 and 45). Patient n°4 and n°8 are more advanced in the pathology, as can be seen from their low ALSFRS scores (28).

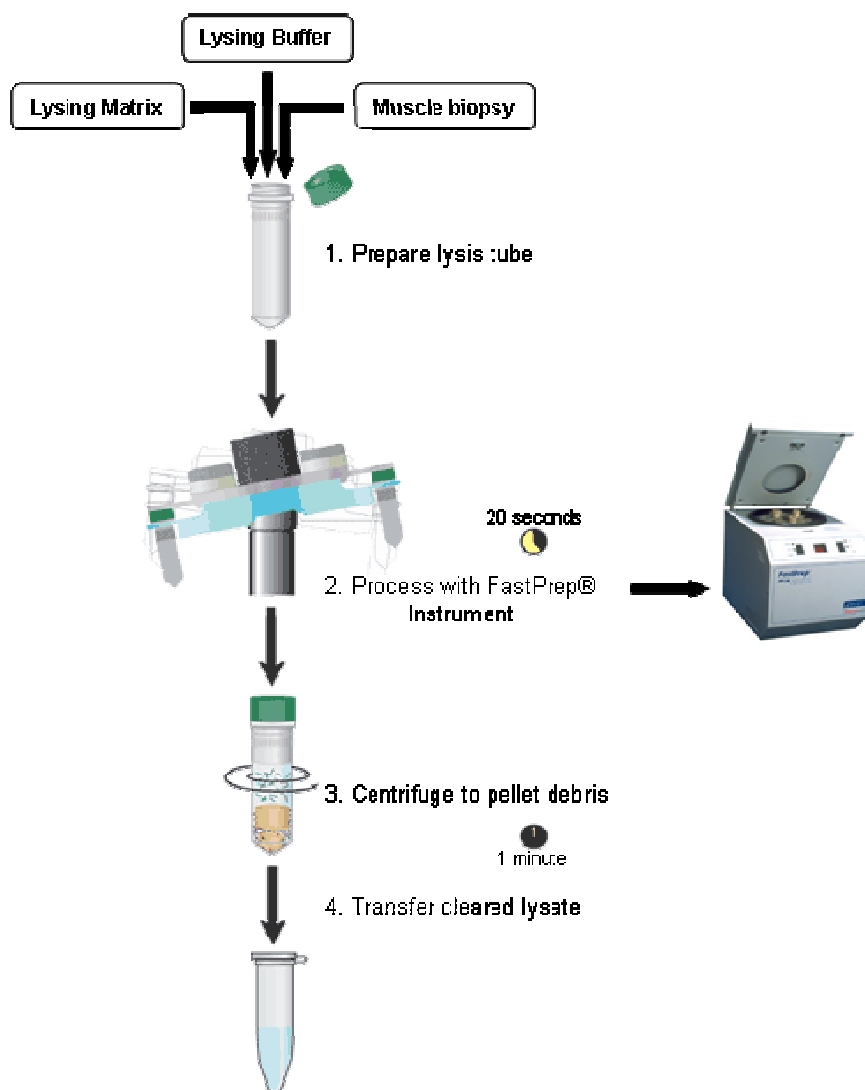


Fig. 10. Total RNA isolation from deltoid muscle biopsies (Adapted from Q-BIOgene website).

The FastPrep system (Q-BIOgene) is characterized by very interesting capabilities. This method replaces cumbersome alternatives such as vortexers, mortar and pestle, sonicators and blenders. It enables rapid and consistent lysis, in a self-contained and easy-to-use system.

RNA isolation protocol: muscle biopsies stored in liquid nitrogen were added frozen on a Bio 101 FastRNA green tube (Q-BIOgene) preloaded with 1 ml Trizol (Invitrogen). Homogenisation (force 6, 40 seconds) was performed in a FastPrep FP120 (Savant). After 5 minutes on ice, 0.2 ml chloroform was added and homogenized by strong vortexing. After 15 minutes centrifugation (full speed, 4°C), total RNA in aqueous phase was precipitated with isopropanol. On average, 38 µg total RNA was isolated and then purified using RNeasy Mini-Spin columns (Qiagen) employing standard protocols provided by the manufacturer: 350 µl RLT buffer was mixed with the total RNA; after adding 250 µl of 70 % ethanol and pipetting up and down, the mixture was loaded onto an RNeasy column, washed and eluted in 50 µl distilled water. Volume was reduced to 11 µl using a speed-vaccum. On average, 24 µg purified total RNA was recovered.

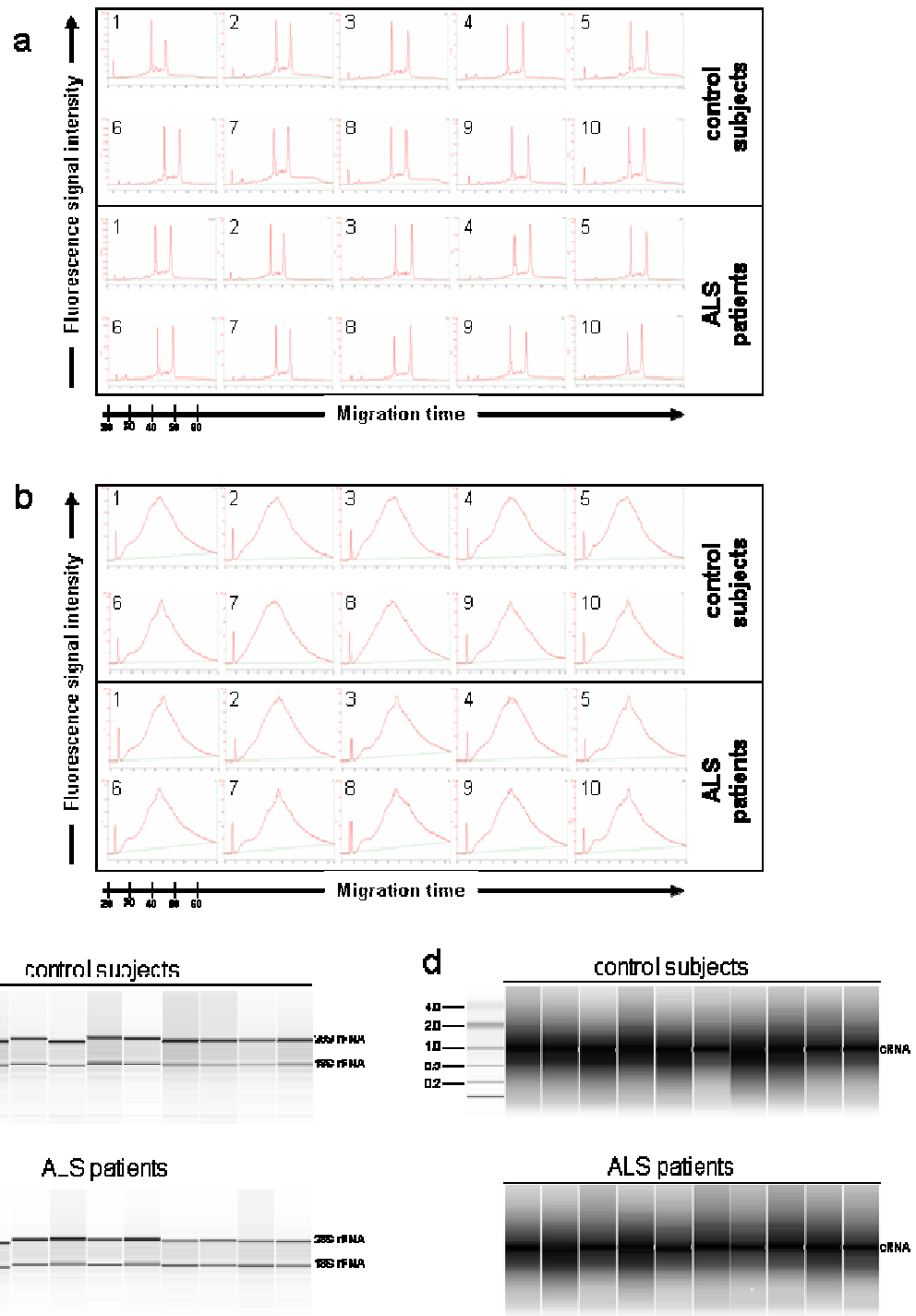


Fig. 11. Total RNA and cRNA target quality assessment.

RNA samples from deltoid muscle biopsies were analysed for their concentration and overall length using the Agilent Bioanalyzer RNA Chip. Fluorescence intensities are plotted against migration time in seconds for total RNA (a) and for cRNA (b). Virtual gels of the total RNA (c) and of the cRNA (d) samples are shown with molecular weight markers indicated on the left of each panel.

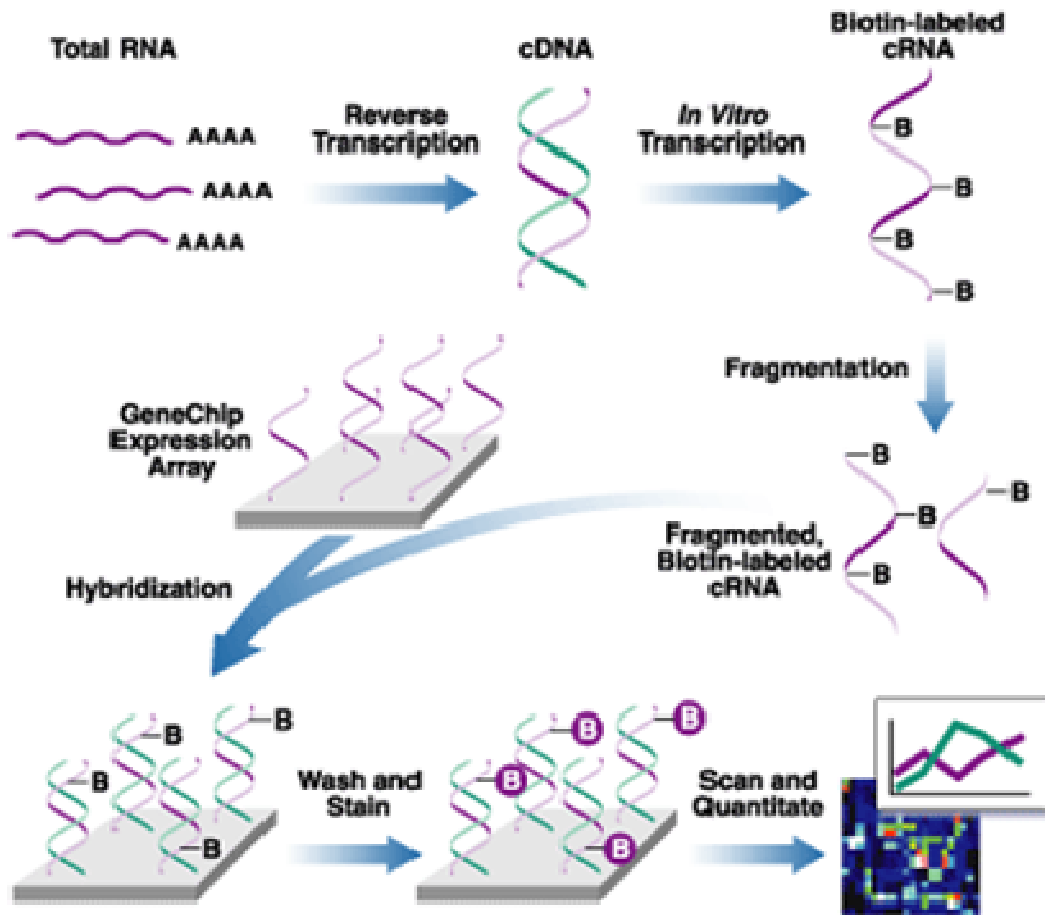


Image courtesy of Affymetrix

Fig. 12. Gene expression assay.

Total RNA is first reverse transcribed using a T7-oligo(dT) promoter primer in the first-strand cDNA synthesis reaction. Following RNase H-mediated second-strand cDNA synthesis, the double-stranded cDNA is purified and used as a template in the subsequent *in vitro* transcription (IVT) reaction. The IVT reaction is carried out in the presence of T7 RNA polymerase and a biotinylated nucleotide analog/ribonucleotide mix for complementary RNA (cRNA) amplification. The biotinylated cRNA targets are then cleaned up, fragmented, and hybridized to GeneChip expression arrays. The hybridized probe array is then washed and stained by a streptavidin-phycoerythrin fluorescent mix. Finally, the probe array is scanned and the obtained image is processed for data quantitation.

3.4. Data quantitation

Data quantitation* is a computer executed task, involving translation of signal* intensities obtained from microarrays into numerical values. It involves making appropriate corrections for values obtained from the sample (signal), while taking into account unwanted intensity values from the microarray surface, or due to differences in probe binding, fluorescence... (commonly referred to as background). In our experiment, images of the scanned GeneChips were stored in data files (.dat) using Microarray Analysis Suite 5.0 (MAS; Affymetrix), and visualized on screen to detect possible abnormalities. Then, MAS created cell intensity files (.cel), by aligning a grid on the image, assigning each cell to an x, y coordinate position, attributing to each cell the corresponding probe set characteristics, and calculating the average intensity of each cell. The .cel files were used to re-analyze data with programs developed in R 1.8.0 [(Ihaka & Gentleman, 1996), <http://www.r-project.org>], a programming language and developer environment for statistical computing and graphics.

3.5. Data normalization

Normalization* is a necessary and useful step, that basically brings readings of all experiments to be on the same scale [(Bolstad et al., 2003) for review]. Actually, all endeavours in microarray data analysis are directed towards the interpretation of inherent differences between samples, often called biological variations. These variations are often obscured by the introduction of erroneous data, often referred as technical or obscuring variations, that are largely the result of differences in experimental procedures such as labelling, hybridization* and washes, disparities between chips and variations in detection procedures. Normalization is the process that corrects for these imbalances in the data. For data normalization, background correction and summarization, we used the Robust Multichip Average (RMA) method (Irizarry et al., 2003), available as part of the R package Affy, which is a part of the Bioconductor project (<http://www.bioconductor.org>). RMA algorithm, based on the

quantile method, seems to perform favorably both in terms of speed, variability and bias, when compared to other algorithms (Bolstad et al., 2003).

3.6. Data mining and modelling

The overall data mining approach we used in our experiment is shown in Fig. 13. Data mining and modelling represents the most important and challenging step in reaching an accurate and meaningful biological conclusion from microarray data. The two central issues involved are the meaningful interpretation of the magnitude of gene expression changes and grouping genes based on some common properties.

Magnitude of gene expression changes and filtering these genes upon this magnitude represents the baseline of data mining and modelling. Filtering methods may use either heuristic algorithms, such as "the fold approach" and "the signal threshold approach", or statistical algorithms, including parametric tests such as the t-test method and the analysis of variance, and nonparametric tests, such as the Wilcoxon-Mann-Whitney test and the Kruskal-Wallis test. Choice among the numerous existing statistical tests depends on certain features including samples number, data distribution, variance equality for each compared group, and number of compared groups. Given the typically heterogeneous nature of the human samples analyzed in our experiment, we chose an heuristic approach by filtering transcripts upon their variance (Fig. 14). Thus, we kept only those transcripts whose standard deviation over all samples was greater than 0.3. Only 3,340 transcripts survived this thresholding operation and served for further analysis.

Gene clustering, the process of grouping together similar genetic elements, is important because it has been noticed that genes sharing a common role in cellular processes, and genes with high degrees of sequence identity tend to be clustered together (Eisen et al., 1998). Clustering approaches can be either unsupervised or supervised. The former method does not use any known reference vectors while the latter is based on a known vector (Brazma & Vilo, 2001; D'Haeseleer et al., 2000). We used the unsupervised Coupled Two-Way Clustering (CTWC) approach (Getz et

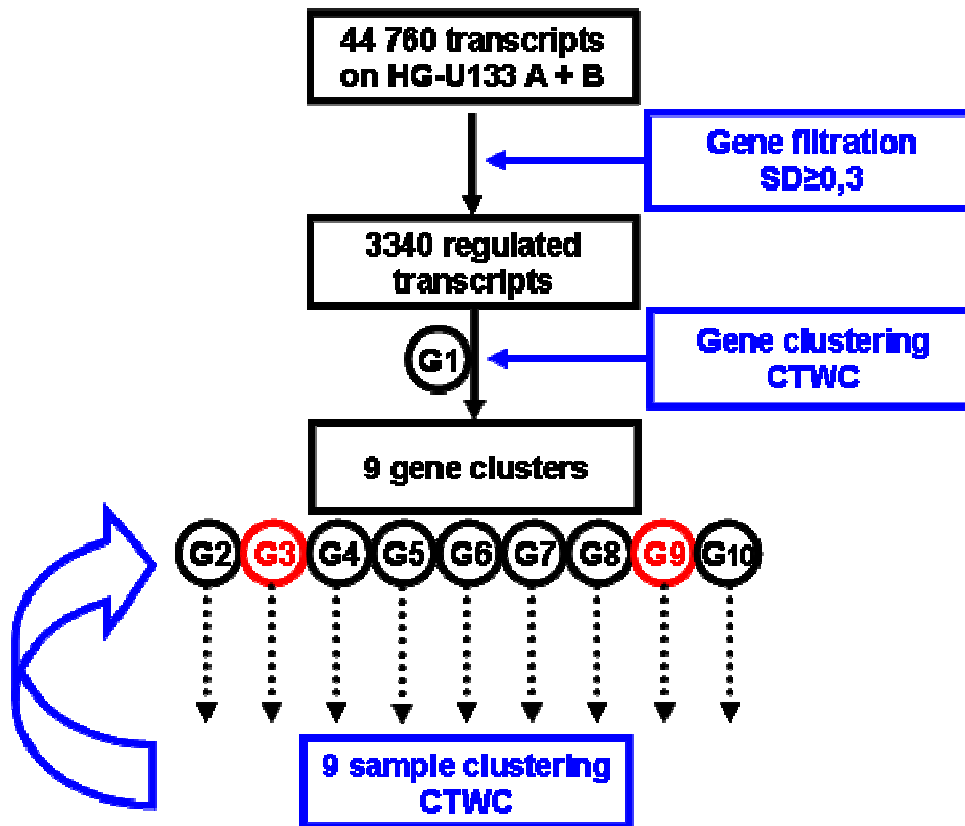


Fig. 13. Data mining.

Data mining steps are indicated in **blue**. We isolated only those transcripts whose standard deviation over all samples is greater than 0.3. We used the Coupled Two-Way Clustering (CTWC) approach to group the 3,340 transcripts that survived this thresholding operation according to their expression profile. This clustering analysis converged to 9 stable gene clusters (G2-G10), and each of these clusters was used to cluster the samples. Two of these sample partitions had clear biological meaning: sample clustering using G3 and sample clustering using G9. Genes contained in G3 and G9 (in **red**) were further analyzed in detail.

1. Gene X signals in the 10 control samples and in the 10 ALS patients

$X_1 - X_2 - X_3 - X_4 - X_5 - X_6 - X_7 - X_8 - X_9 - X_{10} - X_{11} - X_{12} - X_{13} - X_{14} - X_{15} - X_{16} - X_{17} - X_{18} - X_{19} - X_{20}$

2. Standard deviation is calculated for each gene

$$\sigma_x = \frac{\sum (x - \bar{x})^2}{n-1}$$

3. Genes with $\sigma_x > 0,3$ are retained, including:

- genes variable in control subjects but not in ALS patients
- + genes variable in ALS patients but not in control subjects
- + genes variable both in control subjects and in ALS patients
- + genes regulated in ALS patients compared to control subjects

Fig. 14. Steps of gene filtration on standard deviation.

al., 2000; Blatt et al., 1996). The main idea of CTWC is to identify subsets of genes and samples such that when one of these is used to cluster the other, stable and significant partitions emerge. In our hands, the 3,340 regulated transcripts were clustered according to their expression level similarities among the twenty samples analysed (Fig. 15a). This clustering analysis converged to 9 stable gene clusters* (G2-G10) and 25 stable sample clusters (S2-S26). Then, sample clustering was performed using each of the 9 stable gene clusters. Two of these partitions had clear biological meaning: sample clustering using G3 and sample clustering using G9.

3.7. Sample clustering using G3

Clustering analysis using the gene cluster G3 showed that ALS samples are grouped altogether in a stable sample cluster (S6) and clearly separated from the controls (Fig. 15b). Expression of the genes contained in G3 is lower in ALS samples than in controls (Fig. 16a). Indeed, after a Permutation Test* performed on the signal of each gene between ALS samples and controls, the p-value was less than 5% for 15 genes out of the 16 genes analysed (Fig. 17 for a representative example).

3.8. Sample clustering using G9

Clustering analysis using the gene cluster G9 showed that 4 ALS samples are grouped together with all controls in a stable sample cluster (S22) (Fig. 15c). Expression of the genes contained in G9 is higher in the 6 other ALS samples than in sample cluster S22 (Fig. 16b). In addition, after a Spearman correlation test* performed between the signal of each gene for the ten ALS patients and their arm abduction testing score (Fig. 3b), the p-value is less than 5% for 251 genes out of the 274 genes contained in G9 (Fig. 18 for a representative example).

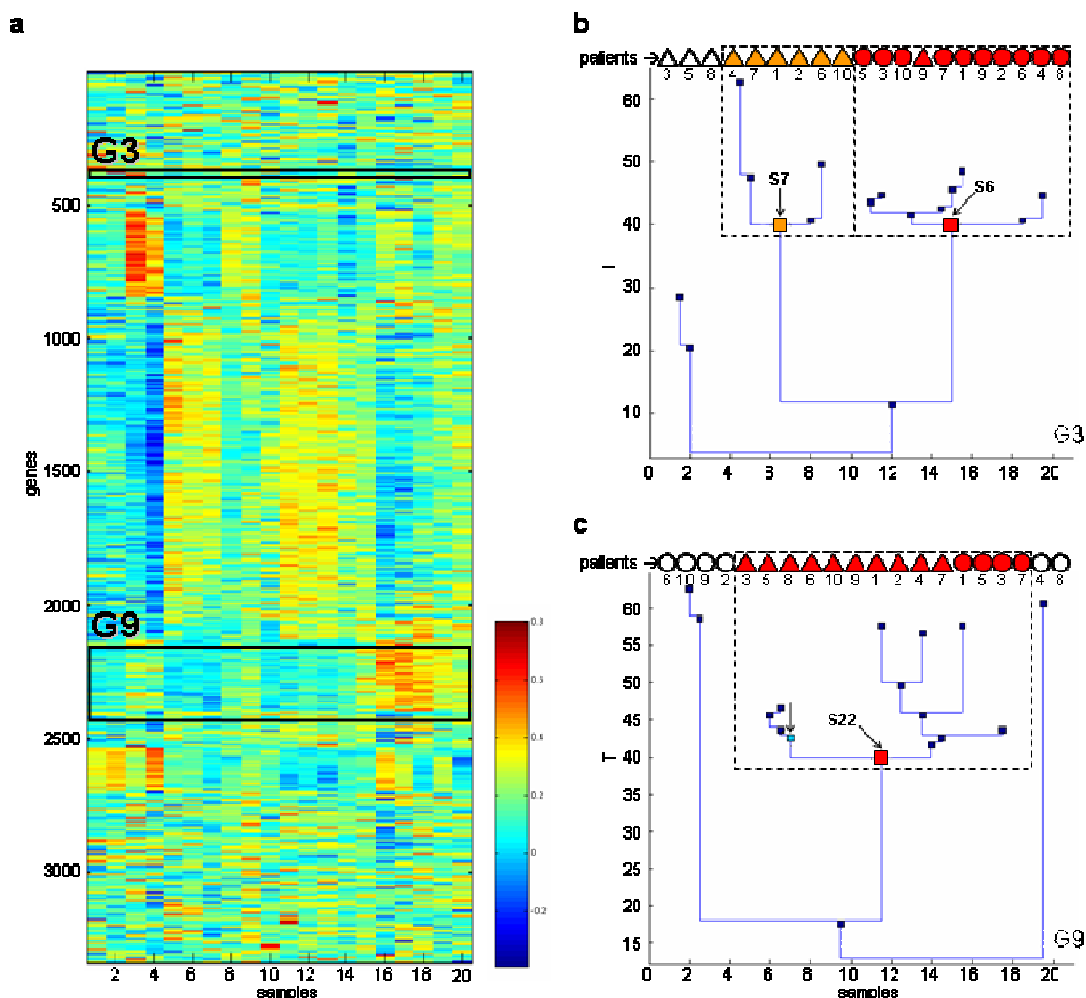


Fig. 15. Unsupervised classification analysis of ALS and control samples.

a. Gene expression matrix* obtained by coupled-two way clustering of gene expression profiles using the 3340 regulated genes. Each row represents a gene and each column represents a sample. Expression data for gene clusters G3 and G9 in all samples are indicated by two black boxes. The color scale represents levels of expression, ranging from low (**blue**) to high expression (**red**) (see color bar on the bottom right).

b and c. Dendrogram* of sample clusters using either gene cluster G3 (b) or gene cluster G9 (c). Sample clusters are shown as boxes. Stable sample clusters* are indicated by an arrow and are coloured either in **light blue**, in **orange** or in **red**. The vertical axis is the "temperature" parameter* T. On the horizontal axis, the samples are ordered according to the dendrogram. The bar at the top of the dendrograms represents the order of the samples, with control samples represented by triangles and ALS samples represented by circles. Patient number is also indicated under the figures.

b. When the genes of G3 are used, clear separation between ALS and control samples is obtained. Stable cluster S6 includes all ALS samples (**red circles**) and stable cluster S7 includes 6 control samples (**orange triangles**).

c. When the genes of G9 are used, all control samples (**red triangles**) together with 4 ALS samples (**red circles**) are clustered in the stable cluster S22. This cluster is clearly separated from the other ALS samples (white circles).

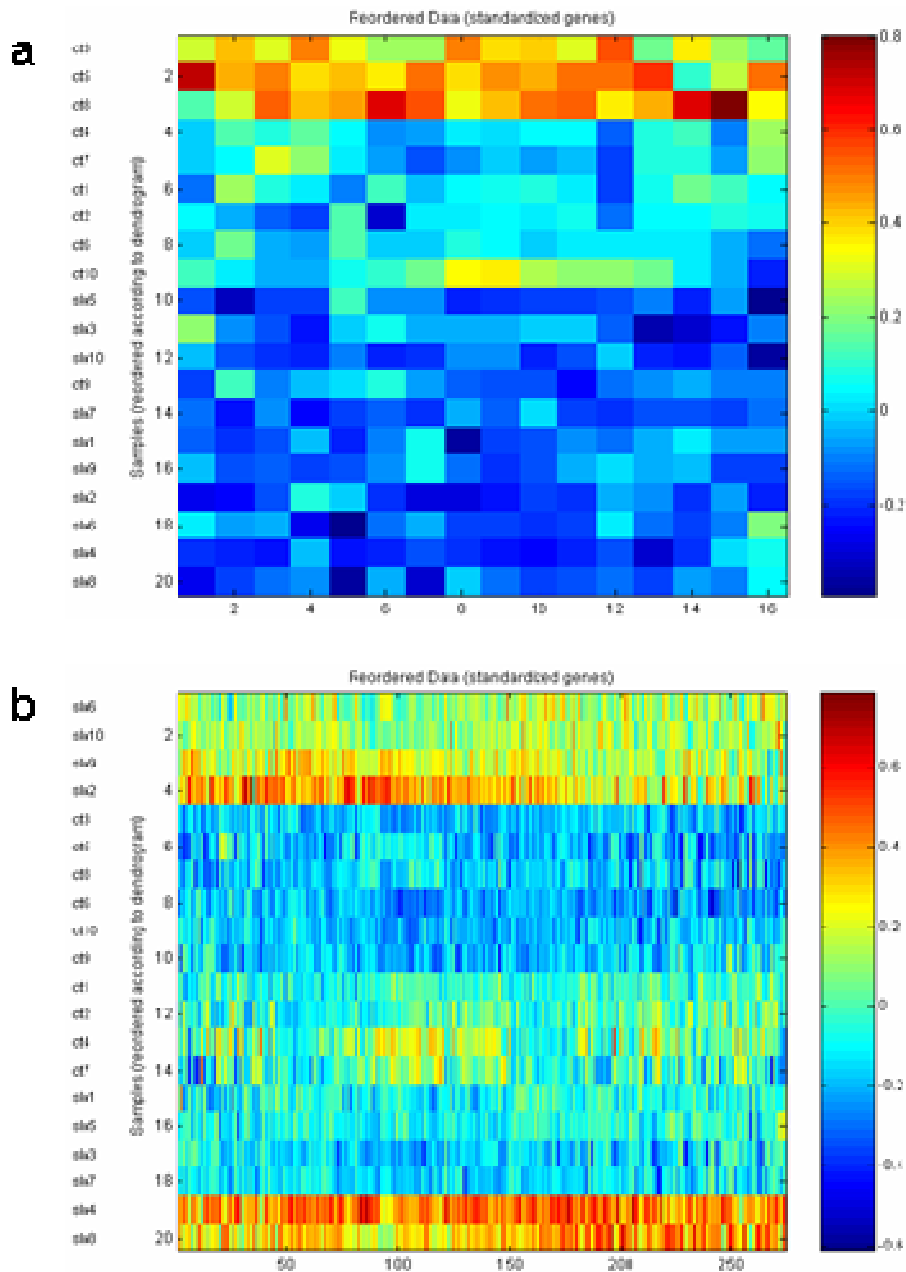


Fig. 16. Reordered data for gene cluster G3 (a) and gene cluster G9 (b).

Each row represents a sample ("sla" for ALS samples and "ct" for control samples) and each column represents a gene (16 genes for G3 and 274 genes for G9). Each row includes the expression levels in a given sample. Samples are ordered as shown in Fig. 15. The color scale represents levels of expression, ranging from low (**blue**) to high expression (**red**) (see color bar on the right).

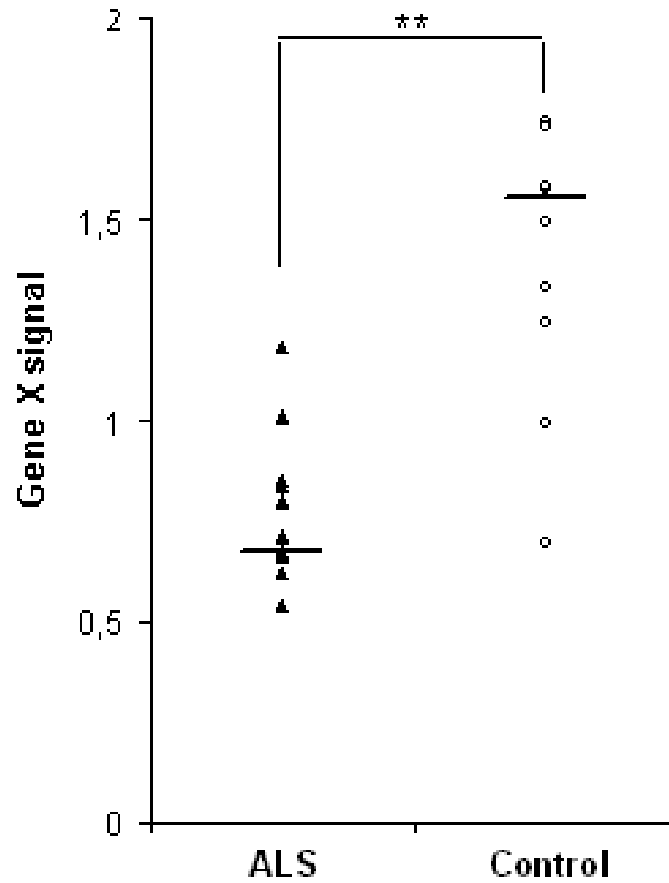


Fig. 17. mRNA levels of gene X (from gene cluster G3).

Gene X signal is represented in each of the ten patients with ALS (dark triangles) and ten control subjects (white circles). ** = $p < 0.01$, with p = permutation test p -value between ALS and control groups. Horizontal bars are mean values.

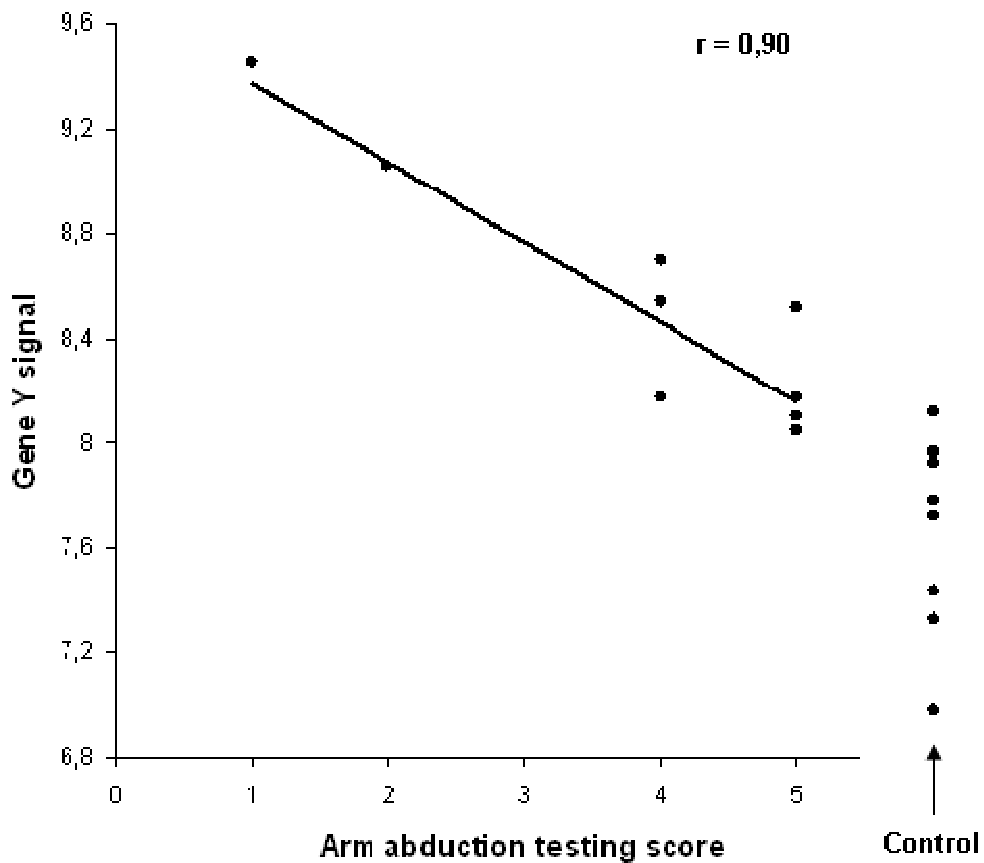


Fig. 18. mRNA levels of gene Y (from gene cluster G9).

On the left, gene Y signal is represented in each of the ten ALS patients compared to their respective arm abduction testing scores. On the right, gene Y signal is figured in each of the ten control subjects and indicated by the arrow. r = Spearman correlation coefficient. Note that the correlation is negative.

3.9. Data annotation

After isolating probesets from gene cluster G3 and G9 that corresponded to known genes (excluding Expressed Sequence Tags* (ESTs)) and grouping them by Unigene* cluster, we performed a manually searching in databases for each probeset to make accurate functional interpretation of our data set (Fig. 19). We centralized useful information in an Excel data sheet as shown in Table. 9. We used the NetAffx web portal (Liu et al., 2003) to retrieve static probeset information and sequence annotations from the HG-U133 Target Databank*. This information included the representative sequence available in GenBank* and RefSeq*, the Unigene cluster identifier for the associated gene (Schuler, 1997), the Online Mendelian Inheritance in ManTM* identifier (OMIM) (McKusick, 1998), the Gene Ontology* (GO) terms (Ashburner et al., 2000; Cheng et al., 2004), and GenMAPP* (Dahlquist et al., 2002) and Kyoto Encyclopedia of Genes and Genomes* (KEGG) (Wixon & Kell, 2000) pathways. Using PubMed, each transcript was assigned with data from the literature that might be relevant in the context of ALS and references of major papers concerning the hit. In addition, we added the corresponding murine sequences and expression data available in the lab on the hit in asymptomatic and symptomatic G86R mice. Finally, we assigned a number ranging from 1 to 4 illustrating the level of relevance for the hit.

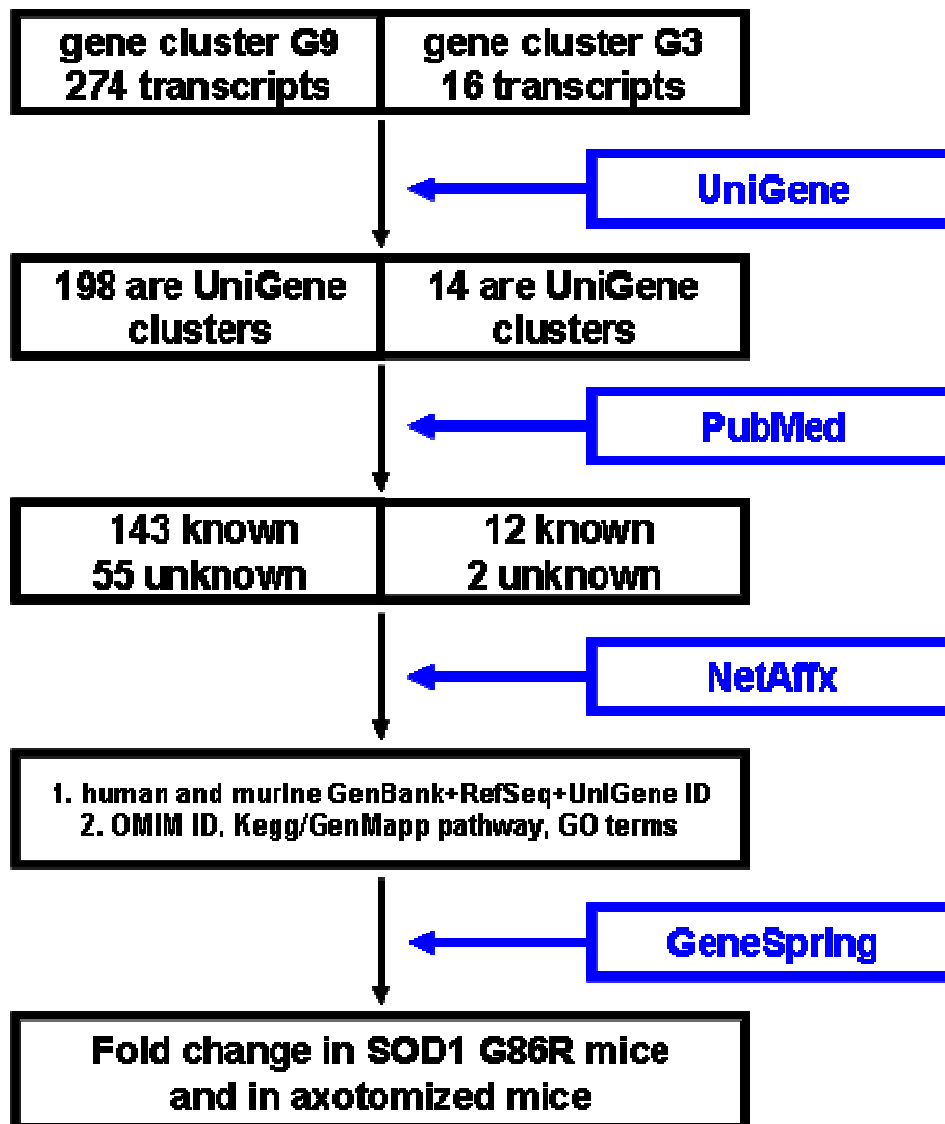


Fig. 19. Data annotation of gene cluster G3 and G9. Databases used are indicated in blue.

Table. 9. Example of data annotation for a gene contained in cluster G9

Gene Title	Gene Symbol	HG-U133 ProbeSet ID	GeneBank ID RefSeq ID	MG-U74Av2 ProbeSet ID	GeneBank ID RefSeq ID	UniGene ID	OMIM ID	Fold Change mSOD1 mice axo mice
calpain 2, (m/II) large subunit	CAPN2	208683_at	M23254 NM_001748	101040_at	D38117	Hs.350899 Mm.6958	114230	nc sod75d up sod105d 1,7 up axo 2,1

GO biology	GO function	GO cellular component	KEGG / GenMAPP Pathway	Notes	PubMed references	Relevance level
proteolysis and peptidolysis	calpain activity calcium ion binding hydrolase activity	intracellular		<p>Nonlysosomal intracellular calcium-activated cysteine protease.</p> <p>CAPN3 is muscle-specific.</p> <p>Calpain inhibitors has provided neuroprotection in models of spinal cord injury.</p> <p>Calpain 1 and calpain 2 mRNA increase in progressive muscular dystrophy and ALS patients.</p> <p>PD150606, a selective calpain antagonist, inhibits KA-induced motor neuron death, but not via calpain inhibition.</p>	<p>Physiol Rev. 2003 83:731-801</p> <p>Brain Res Brain Res Rev. 2003 42:169-85</p> <p>Physiol Rev. 2000 80:1215-65</p> <p>J Neurol Sci. 1998 155:163-9</p> <p>Neuropharmacology. 2002 42:706-13</p>	1

ID: identifier. up/nc/down: gene up-regulated/not regulated/down-regulated compared to the control group. sod: SOD1 G86R mice. axo: axotomized mice. 75/105d: 75/105 days old.

3.10. Microarray expression data validation

Three genes from gene cluster G9 and G3 were arbitrarily selected to verify microarray expression data. The expression of these genes was examined by QPCR in 12 of the control and ALS samples. The same RNA sample used for microarray experiments was analysed. The 18S ribosomal RNA was used as control for sample loading in both microarray and QPCR experiments. The relative amounts of the three mRNAs evaluated by QPCR strongly correlated with the microarray-based measurements (Fig. 20). Besides, some genes contained in these clusters are already known to be regulated in ALS, including calpain 2 [(Ueyama et al., 1998), Table. 9 and Fig. 21] and thioredoxin (Malaspina et al., 2001; Ogawa et al., 1997). These data further supports the reliability of the experimental approach.

3.11. An example of application: retinoic acid metabolism in ALS

Surprisingly, several hits emerging from our data analysis were linked to retinoic acid (RA) metabolism (Fig. 22). Among them, the retinaldehyde dehydrogenase 2 (RALDH-2), that convert the retinal derived from ingested β -carotene to RA, the RA binding protein I (CRABP-I), that binds RA in the cytoplasm, and P450RAI (CYP26), a cytochrome P450 enzyme that specifically metabolizes RA and channels RA towards catabolic pathways, are differentially regulated in ALS muscle according to our GeneChip experiment (Fig. 23). Although the biological meaning of these regulations in an ALS context needs exhaustive research, it is worth emphasizing that very early studies already reported nerve lesions induced by defects in the retinoid signalling pathway (Irving & Richards, 1938; Hughes et al., 1924). Moreover, retinoid-deficient rats exhibit phenotypes reminiscent of those observed in ALS patients, including accumulation of neurofilament and vacuolar lesions in motor neurons in the spinal cord, loss of motor neurons and increased

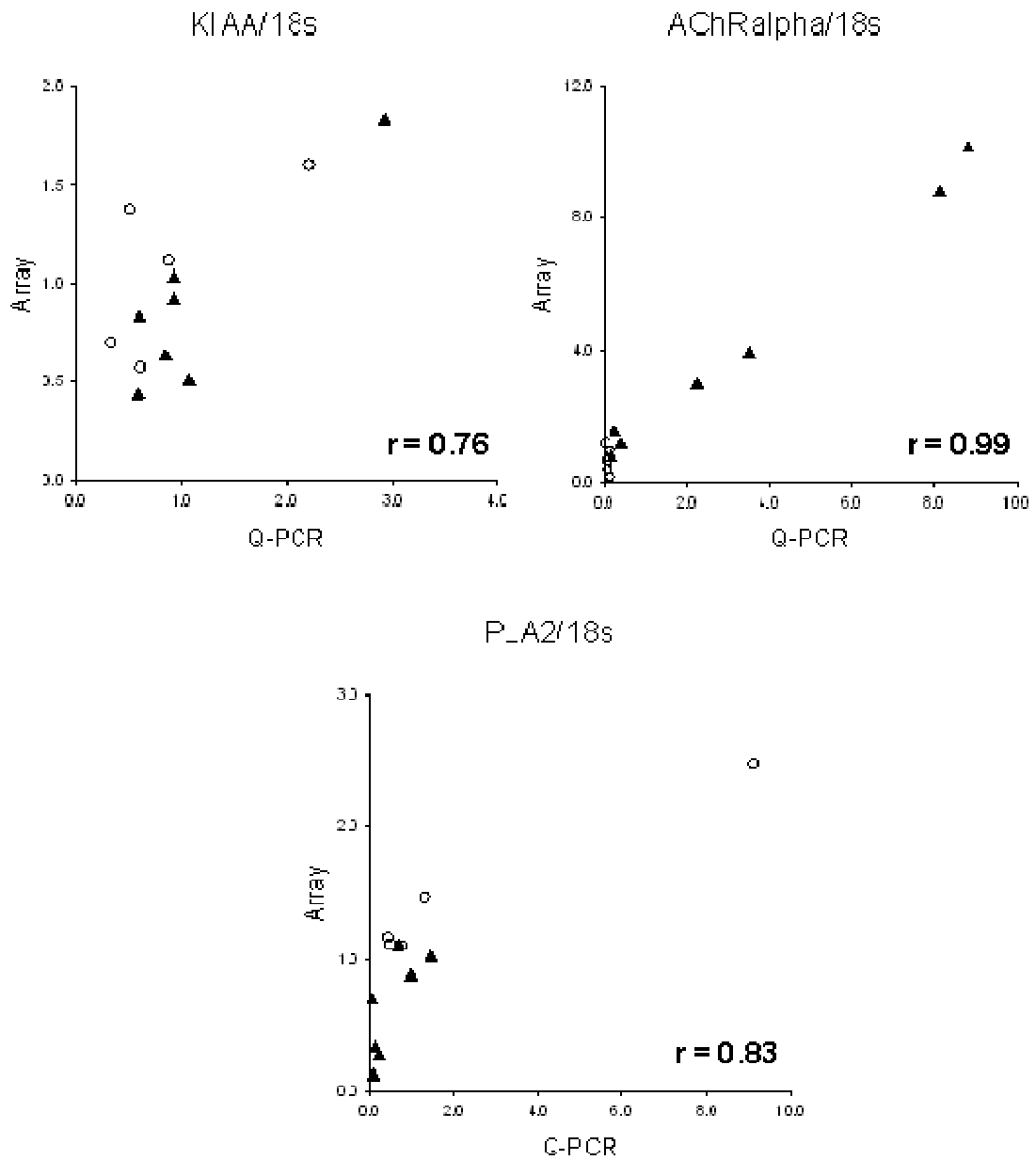


Fig. 20. Correlation analysis of data obtained from GeneChips and quantitative PCR (Q-PCR).

The ratio mRNA to 18S rRNA was determined for both GeneChips and QPCR experiments. The relative values for each gene from 7 ALS (dark triangles) and 5 control samples (white circles) are shown. r = Pearson correlation* coefficient.

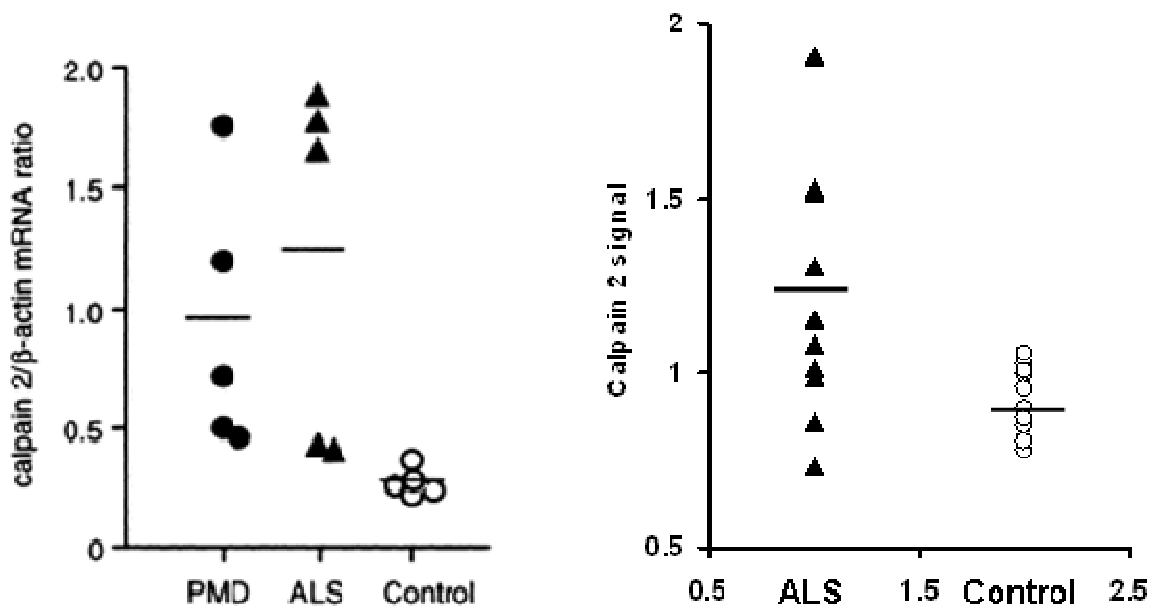


Fig. 21. Calpain 2 contained in cluster G9 is already known to be regulated in ALS muscle.

On the left, mRNA levels of calpain 2 (normalized by mRNA level of β -actin) measured by Q-PCR in biceps muscle biopsy from control subjects (white circles) and from patients with progressive muscular dystrophy (PMD - dark circles) and ALS (white circles). Results from Ueyama et al., 1998.

On the right, calpain 2 signal measured in our GeneChip experiment is represented in each of the ten patients with ALS (dark triangles) and ten control subjects (white circles). Horizontal bars are mean values.

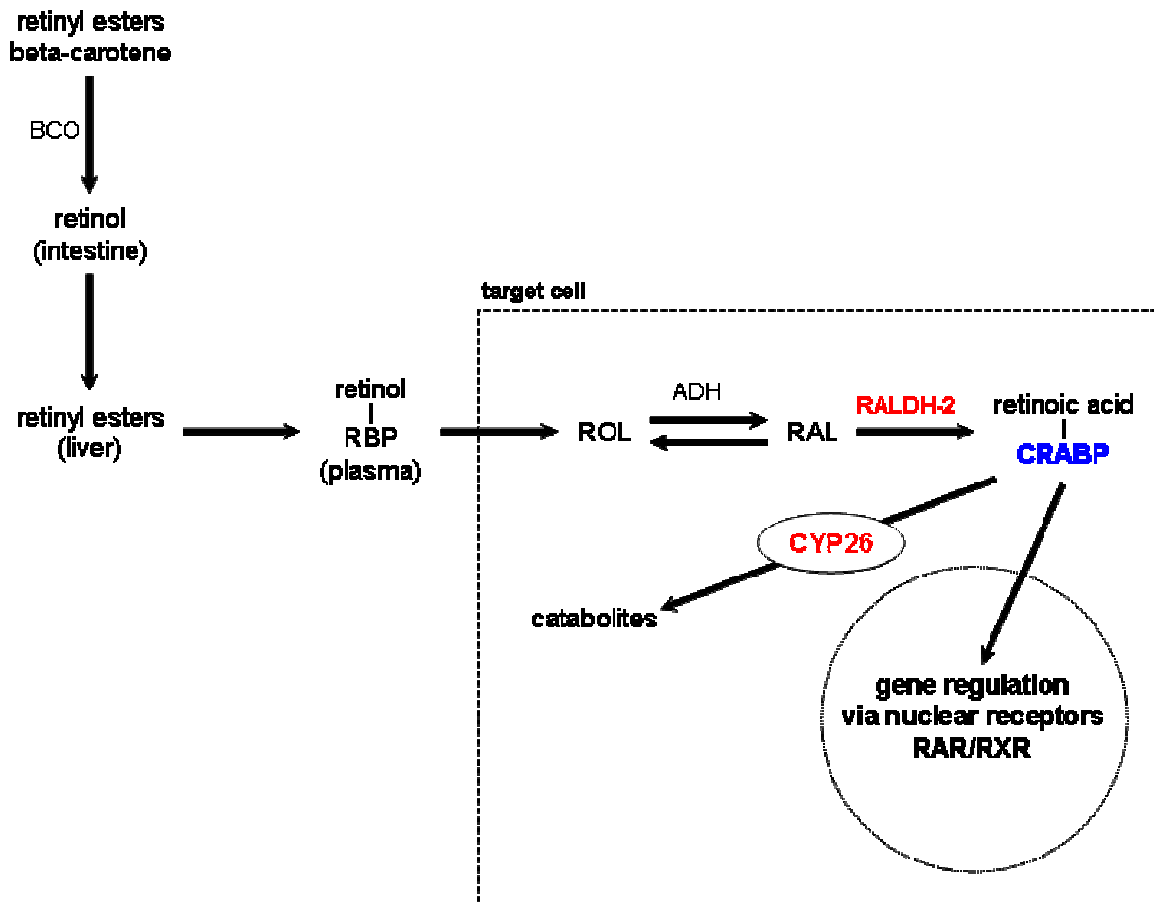


Fig. 22. Retinoic acid metabolic pathway.

Retinylesters and β -carotene are ingested and converted to retinol (ROL) in the intestine by β,β -carotene-15,15'-oxygenase (BCO) activity. ROL is then reconverted to retinyl esters for storage, mainly in the liver. Demand for ROL results in the release of ROL that is bound to plasma retinol-binding protein (RBP) from the liver. Conversion of ROL to the active forms of retinoic acid (RA) occurs in many different tissues, including skeletal muscle. RA is converted from ROL precursor by a conversion to retinaldehyde (RAL) by alcohol dehydrogenase (ADH) activity and subsequently to RA by retinaldehyde dehydrogenase 2 (RALDH-2) activity. RA is bound in the cytoplasm by cellular RA-binding proteins (CRABP). RA will act on RA nuclear receptors (RARs and RXRs) to activate the expression of RA responsive genes. This activity can be blocked if RA-metabolizing enzymes, such as P450RAI (CYP26), are present, channeling RA towards a catabolic pathway. In our GeneChip experiment, genes are indicated **in red** when induced and **in blue** when repressed.

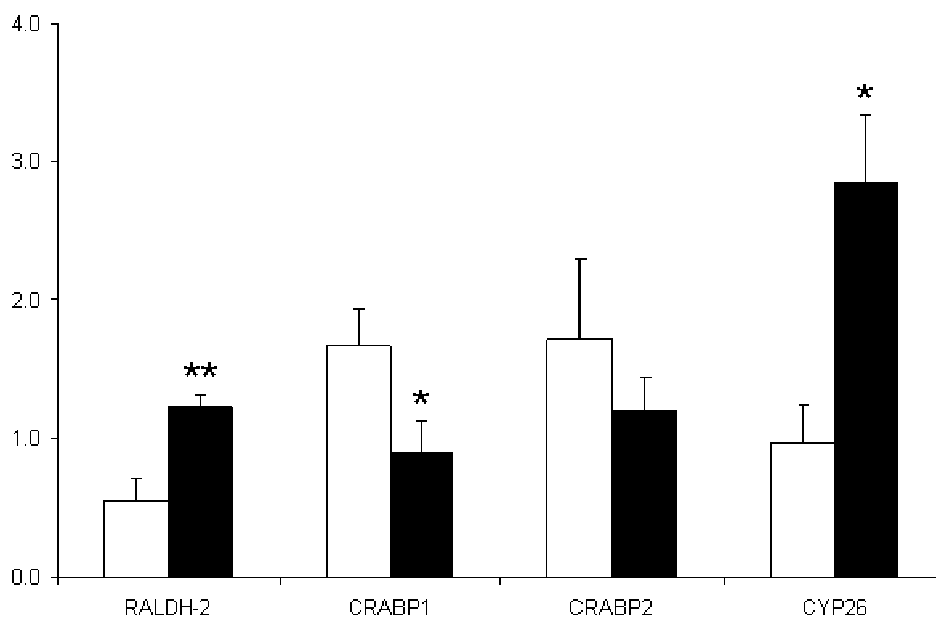


Fig. 23. Regulated genes involved in retinoic acid metabolic pathway.

Retinaldehyde dehydrogenase 2 (RALDH-2), cellular retinoic acid-binding proteins (CRABP1 and 2) and P450RAI (CYP26) signals are indicated as mean values in the ten patients with ALS (in dark) and ten control subjects (in white). ** = $p < 0.01$, * = $p < 0.05$ with $p =$ student test p -value between ALS and control groups.

reactive astrocytosis in the lumbar cord (Corcoran et al., 2002). In patients suffering from spontaneous motor neuron disease, defects in the retinoid signalling pathway were also found (Corcoran et al., 2002), including decreased *raldh-2* expression in motor neurons of diseased spinal cord. These results suggest that a defect in the retinoid signalling pathway might have a role in ALS pathogenesis.

Although serum and plasma levels of vitamin A are normal in ALS (Molina et al., 1999; Iwasaki et al., 1995; Bonnefont-Rousselot et al., 2000), several links between retinoic acid metabolism and ALS pathogenesis might be speculated. Vitamin A and analogues are potential antioxidant factors, notably through their characteristic chemical and physical properties [(Demmig-Adams & Adams, 2002) for review]. It has been shown that retinol, retinol acetate, RA, retinol palmitate, and retinal protect neuronal membranes from lipid peroxidation by free radicals (Das, 1989). Moreover, RA inhibits hydrogen peroxide-induced apoptosis via suppression of c-fos/c-jun expression and c-Jun N-terminal kinase activation in glomerular cells (Kitamura et al., 2002). It has also been shown that binding of the peroxisome proliferator-activated receptor to the SOD1 promoter was increased by treatment with RA and 9-cis RA (Yoo et al., 1999). Therefore, RA might act coordinately with others molecular effectors in preventing the increased oxidative stress in ALS muscle. RA is also widely described as an active player in the regeneration of several tissues and organs [(Maden & Hind, 2003) for review]. Notable examples include stimulation of neurite outgrowth, via induction of RA synthesis by nerve growth factor (Corcoran & Maden, 1999); regulation of cell growth and differentiation of the vascular system [(Gardner & Chen, 1999; Miano & Berk, 2000; Neuville et al., 2000) for review], possibly via stimulation of VEGF release in smooth muscle cells (Tanabe et al., 2004); and multiple implications in myogenic cascades (Busson et al., 2005; Mic & Duester, 2003; Shin et al., 2000; Georgiades & Brickell, 1997; Larrain et al., 1997; Xiao et al., 1995). Therefore, alterations in muscular RA pathway might reflect the differentiation or de-differentiation component of the ALS disease. Finally, it is also worth emphasizing that muscle UCP3 expression is up-regulated in mice following dietary vitamin A supplementation and acute RA treatment (Felipe et al., 2003). These findings together with our present data presage an important role of the retinoid signalling pathway in ALS pathogenesis., and research in this field is currently in progress in our laboratory.

Conclusion and Perspectives

The microarray approach we conducted in muscle biopsies from ALS patients has enabled the creation of a database in which genes are systematically profiled to constitute a molecular identity card of skeletal muscle affected by the disease. We are currently exploiting this database following three main lines of research.

New progression markers of ALS

Markers of ALS progression need tight correlation between the measured signal intensity and clinical measures as motor strength or vital capacity, observer-independent quantification, strong and linear changes in signal intensity during the disease progression, a procedure allowing serial examinations in the same patient non-invasively, and ideally a protocol that is cheap and rapidly applied to a large number of subjects. Genes included in cluster G9 fulfill at least several of these criteria: their signal intensities correlate with a testing score, gene expression measurements are relatively independent from the bench scientist and are easily applied to a large number of samples. Similarly, Nogo-A is a potential marker of ALS progression, since protein levels of Nogo-A dramatically increase in ALS skeletal muscle and correlate with the ALSFRS scale. However, these preliminary markers need to be confirmed in a larger patient group and followed during longitudinal clinical studies.

New diagnostic tools for ALS

In contrast to progression markers, early diagnostic markers need specificity of the observed signal for ALS. Moreover, they also need high sensitivity so that patients in early or ideally in preclinical disease stages may be screened. In this context, genes included in cluster G3 and UCP3 are interesting candidates insofar as their expression may appear highly and precociously regulated, as compared either to normal subjects or to patients affected by other neurological or muscular diseases. However, comparisons with many other ALS-like syndromes are still needed to affirm specificity of these markers. In addition, diagnostic procedures need to be refined, standardized and financially optimized to become a routine diagnostic tool. Such specificity and sensitivity explorations are already launched both in a clinical context,

in collaboration with the service of Prof. Vincent Meininger (*Hôpital de la Pitié-Salpêtrière, Paris*), and in our laboratory. In particular, we have performed GeneChips analyses of skeletal muscle from G86R mice and from axotomized mice that modelize acute denervation. These investigations will give us access to very precocious genetic modifications that might appear prior to overt symptoms. They will also contribute to identify gene regulations accompanying neurogenic atrophy, and will enable us to cross results between ALS patients and a widely studied model of ALS. In addition, "Meta-analyses" using the existing and ever increasing pool of gene microarray databases (easily accessible via the Gene Expression Omnibus* data repository of the National Center for Biotechnology Information) would enable us to exclude genes not related specifically to ALS. For example, microarray databases are available on transcriptional adaptations underlying skeletal muscle atrophy, which is a common response to many disorders (Jagoe et al., 2002; Stevenson et al., 2003; Lecker et al., 2004; Kostrominova et al., 2005).

New genes involved in ALS pathogenesis

Exploring our database might shed light on the pathogenic mechanisms of ALS. Particularly, we identified alterations in the RA signalling pathway that are now under investigation. Indeed, we are currently analyzing the expression levels of RA signalling-linked genes [(Balmer & Blomhoff, 2002) for classification] in our GeneChips murine and human databases. In parallel, we are measuring RA concentration in G86R muscle and liver, and we are testing the effects of a RA-enriched diet on G86R mice.

As far as Nogo-A is concerned, many studies are currently performed to explore its involvement in ALS pathogenesis. In collaboration with the laboratory of Prof. Martin Schwab (Brain Research Institute, Zurich), we showed that G86R/Nogo-A^{-/-} double transgenics, generated by crossing G86R and Nogo-A knockout mice (Simonen et al., 2003), survived about 20% more than G86R/Nogo-A^{+/+} animals (Jokic et al., manuscript in preparation). In collaboration with the laboratory of Prof. Markus Rüegg (Biozentrum, Basel), we also observed that *in vivo* overexpression of Nogo-A by intramuscular transfection revealed several morphological abnormalities at the neuromuscular junction (NMJ) level, including disappearance of pre-synaptic terminals, as well as fragmentation of post-synaptic structures (Jokic et al.,

manuscript in preparation). These findings are reminiscent of that observed in ALS patients and in mouse models of ALS (Yoshihara et al., 1998; Fischer et al., 2004), and suggest that induction of Nogo-A, as observed in ALS muscle, might interfere with nerve sprouting and synapse plasticity. Such changes might induce "destabilization" of the NMJ and might, at least in part, contribute to the characteristic denervation of ALS. This quite novel concept of "myogenic" insult in ALS, provoked by Nogo-A induction or other abnormalities in local retrograde signaling from the skeletal muscle, is in accordance with the axonal dying-back model already defended by some authors [(Coleman & Perry, 2002; Glass, 2004; Santos & Caroni, 2003) for review]. In this model, upstream events specific to ALS would lead to a progressive synaptic weakening, which in turn would result in denervation long before the onset of the clinical phase and the death of motor neurons (Frey et al., 2000). This is also consistent with reports of early transmission defects in a dog model of ALS (Pinter et al., 1995) and in ALS patients (Maselli et al., 1993).

The hypothesis supporting distal injury in ALS could help to the development of novel therapeutic strategies. This idea has already been tested by stabilizing the NMJ in mouse models of congenital muscular dystrophy (Moll et al., 2001; Bentzinger et al., 2005). This kind of approach is also explored in our laboratory by crossing G86R mice with animals overexpressing agrin, a key regulator of the formation of postsynaptic structures at the NMJ (collaboration with the laboratory of Prof. Markus Rüegg). Another strategy would be early treatment of peripheral synapses by regenerative agents. These may include the monoclonal antibody raised against Nogo-A, IN-1 (Merkler et al., 2001), or neurotrophic factors such as IGF-1 (Dobrowolny et al., 2005; Kaspar et al., 2003), GDNF (Sagot et al., 1996) and neurotrophin-3 (Haase et al., 1997). Targeting motor neuron axon would also constitute another interesting approach. Actually, it is surprising that most strategies to combat ALS focussing on neuronal cell bodies have little effect (Sagot et al., 1996; Sagot et al., 1995). In contrast, strategies that protect motor neuron axons appear more efficient (Williamson et al., 1998; Williamson & Cleveland, 1999; Couillard-Despres et al., 1998). Factors involved in axonal degeneration may be distinct from those inducing death of neuronal cell body (Deckwerth & Johnson, 1994; Finn et al., 2000; Sagot et al., 1995). Therefore, approaches targeting distal injury might have been hindered by a lack of appropriate leads to follow (Coleman & Perry, 2002). In

this respect, our databases will hopefully fill this gap in the search of new targetable molecules.

In conclusion, this PhD work may be considered as a bold and daring step in ALS research. Indeed, the concept making skeletal muscle an active player in ALS goes against the general trend that largely focus on nervous system. In this context, the microarray approach was an interesting tool, since it allowed us to study this quite unexplored pathway in an unbiased way, irrespective of any preconception. Moreover, knowing the expression pattern of a large number of genes has given us a broader and more complete picture of the cellular processes occurring in skeletal muscle affected by ALS. Hopefully, such studies will engage new efforts to exploit this unending source of information.

References

- Abe, K., Yorifuji, S. & Nishikawa, Y. 1993. Reduced isotope uptake restricted to the motor area in patients with amyotrophic lateral sclerosis. *Neuroradiology*, **35**, 410-1.
- Acevedo, L., Yu, J., Erdjument-Bromage, H., Miao, R. Q., Kim, J. E., Fulton, D., Tempst, P., Strittmatter, S. M. & Sessa, W. C. 2004. A new role for Nogo as a regulator of vascular remodeling. *Nat Med*, **10**, 382-8.
- Acsadi, G., Anguelov, R. A., Yang, H., Toth, G., Thomas, R., Jani, A., Wang, Y., Ianakova, E., Mohammad, S., Lewis, R. A. & Shy, M. E. 2002. Increased survival and function of SOD1 mice after glial cell-derived neurotrophic factor gene therapy. *Hum Gene Ther*, **13**, 1047-59.
- Aitkens, S., Lord, J., Bernauer, E., Fowler, W. M., Jr., Lieberman, J. S. & Berck, P. 1989. Relationship of manual muscle testing to objective strength measurements. *Muscle Nerve*, **12**, 173-7.
- Al Halabiah, H., Delezoide, A. L., Cardona, A., Moalic, J. M. & Simonneau, M. 2005. Expression pattern of NOGO and NgR genes during human development. *Gene Expr Patterns*, **5**, 561-8.
- Al-Chalabi, A., Andersen, P. M., Nilsson, P., Chioza, B., Andersson, J. L., Russ, C., Shaw, C. E., Powell, J. F. & Leigh, P. N. 1999. Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. *Hum Mol Genet*, **8**, 157-64.
- al-Chalabi, A., Enayat, Z. E., Bakker, M. C., Sham, P. C., Ball, D. M., Shaw, C. E., Lloyd, C. M., Powell, J. F. & Leigh, P. N. 1996. Association of apolipoprotein E epsilon 4 allele with bulbar-onset motor neuron disease. *Lancet*, **347**, 159-60.
- Al-Chalabi, A., Scheffler, M. D., Smith, B. N., Parton, M. J., Cudkowicz, M. E., Andersen, P. M., Hayden, D. L., Hansen, V. K., Turner, M. R., Shaw, C. E., Leigh, P. N. & Brown, R. H., Jr. 2003. Ciliary neurotrophic factor genotype does not influence clinical phenotype in amyotrophic lateral sclerosis. *Ann Neurol*, **54**, 130-4.
- Alizadeh, A. A., Eisen, M. B., Davis, R. E., Ma, C., Lossos, I. S., Rosenwald, A., Boldrick, J. C., Sabet, H., Tran, T., Yu, X., Powell, J. I., Yang, L., Marti, G. E., Moore, T., Hudson, J., Jr., Lu, L., Lewis, D. B., Tibshirani, R., Sherlock, G., Chan, W. C., Greiner, T. C., Weisenburger, D. D., Armitage, J. O., Warnke, R., Levy, R., Wilson, W., Grever, M. R., Byrd, J. C., Botstein, D., Brown, P. O. & Staudt, L. M. 2000. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*, **403**, 503-11.
- Andersen, H. & Jakobsen, J. 1997. A comparative study of isokinetic dynamometry and manual muscle testing of ankle dorsal and plantar flexors and knee extensors and flexors. *Eur Neurol*, **37**, 239-42.

- Andres, P. L., Finison, L. J., Conlon, T., Thibodeau, L. M. & Munsat, T. L. 1988. Use of composite scores (megascoring) to measure deficit in amyotrophic lateral sclerosis. *Neurology*, **38**, 405-8.
- Andres, P. L., Hedlund, W., Finison, L., Conlon, T., Felmus, M. & Munsat, T. L. 1986. Quantitative motor assessment in amyotrophic lateral sclerosis. *Neurology*, **36**, 937-41.
- Appel, V., Stewart, S. S., Smith, G. & Appel, S. H. 1987. A rating scale for amyotrophic lateral sclerosis: description and preliminary experience. *Ann Neurol*, **22**, 328-33.
- Arsenijevic, D., Onuma, H., Pecqueur, C., Rimbault, S., Manning, B. S., Miroux, B., Couplan, E., Alves-Guerra, M. C., Goubert, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S. & Ricquier, D. 2000. Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat Genet*, **26**, 435-9.
- Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M. & Sherlock, G. 2000. Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet*, **25**, 25-9.
- Azzouz, M., Ralph, G. S., Storkebaum, E., Walmsley, L. E., Mitrophanous, K. A., Kingsman, S. M., Carmeliet, P. & Mazarakis, N. D. 2004. VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature*, **429**, 413-7.
- Balmer, J. E. & Blomhoff, R. 2002. Gene expression regulation by retinoic acid. *J Lipid Res*, **43**, 1773-808.
- Bandtlow, C. E., Dlaska, M., Pirker, S., Czech, T., Baumgartner, C. & Sperk, G. 2004. Increased expression of Nogo-A in hippocampal neurons of patients with temporal lobe epilepsy. *Eur J Neurosci*, **20**, 195-206.
- Barbeito, L. H., Pehar, M., Cassina, P., Vargas, M. R., Peluffo, H., Viera, L., Estevez, A. G. & Beckman, J. S. 2004. A role for astrocytes in motor neuron loss in amyotrophic lateral sclerosis. *Brain Res Brain Res Rev*, **47**, 263-74.
- Bates, G. 2000. Huntington's disease. In reverse gear. *Nature*, **404**, 944-5.
- Beal, M. F., Ferrante, R. J., Browne, S. E., Matthews, R. T., Kowall, N. W. & Brown, R. H., Jr. 1997. Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis. *Ann Neurol*, **42**, 644-54.
- Beer, D. G., Kardia, S. L., Huang, C. C., Giordano, T. J., Levin, A. M., Misek, D. E., Lin, L., Chen, G., Gharib, T. G., Thomas, D. G., Lizyness, M. L., Kuick, R., Hayasaka, S., Taylor, J. M., Iannettoni, M. D., Orringer, M. B. & Hanash, S. 2002. Gene-expression profiles predict survival of patients with lung adenocarcinoma. *Nat Med*, **8**, 816-24.

- Bentzinger, C. F., Barzaghi, P., Lin, S. & Ruegg, M. A. 2005. Overexpression of mini-agrin in skeletal muscle increases muscle integrity and regenerative capacity in laminin-alpha2-deficient mice. *Faseb J*, **19**, 934-42.
- Beuche, W., Yushchenko, M., Mader, M., Maliszewska, M., Felgenhauer, K. & Weber, F. 2000. Matrix metalloproteinase-9 is elevated in serum of patients with amyotrophic lateral sclerosis. *Neuroreport*, **11**, 3419-22.
- Bittner, M., Meltzer, P., Chen, Y., Jiang, Y., Seftor, E., Hendrix, M., Radmacher, M., Simon, R., Yakhini, Z., Ben-Dor, A., Sampas, N., Dougherty, E., Wang, E., Marincola, F., Gooden, C., Lueders, J., Glatfelter, A., Pollock, P., Carpten, J., Gillanders, E., Leja, D., Dietrich, K., Beaudry, C., Berens, M., Alberts, D. & Sondak, V. 2000. Molecular classification of cutaneous malignant melanoma by gene expression profiling. *Nature*, **406**, 536-40.
- Blatt, M., Wiseman, S. & Domany, E. 1996. Superparamagnetic clustering of data. *Physical Review Letters*, **76**, 3251-3254.
- Block, W., Karitzky, J., Traber, F., Pohl, C., Keller, E., Mundegar, R. R., Lamerichs, R., Rink, H., Ries, F., Schild, H. H. & Jerusalem, F. 1998. Proton magnetic resonance spectroscopy of the primary motor cortex in patients with motor neuron disease: subgroup analysis and follow-up measurements. *Arch Neurol*, **55**, 931-6.
- Bogdanov, M., Brown, R. H., Matson, W., Smart, R., Hayden, D., O'Donnell, H., Flint Beal, M. & Cudkovicz, M. 2000. Increased oxidative damage to DNA in ALS patients. *Free Radic Biol Med*, **29**, 652-8.
- Bolstad, B. M., Irizarry, R. A., Astrand, M. & Speed, T. P. 2003. A comparison of normalization methods for high density oligonucleotide array data based on variance and bias. *Bioinformatics*, **19**, 185-93.
- Bonnefont-Rousselot, D., Lacomblez, L., Jaudon, M., Lepage, S., Salachas, F., Bensimon, G., Bizard, C., Doppler, V., Delattre, J. & Meininger, V. 2000. Blood oxidative stress in amyotrophic lateral sclerosis. *J Neurol Sci*, **178**, 57-62.
- Borecky, J., Maia, I. G. & Arruda, P. 2001. Mitochondrial uncoupling proteins in mammals and plants. *Biosci Rep*, **21**, 201-12.
- Boss, O., Hagen, T. & Lowell, B. B. 2000. Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. *Diabetes*, **49**, 143-56.
- Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P. & Giacobino, J. P. 1997. Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett*, **408**, 39-42.
- Brazma, A. & Vilo, J. 2001. Gene expression data analysis. *Microbes Infect*, **3**, 823-9.
- Brody, J. A., Stanhope, J. M. & Kurland, L. T. 1975. Patterns of Amyotrophic Lateral Sclerosis and Parkinsonism-Dementia on Guam. *Contemp Neurol Ser*, **12**, 45-70.

- Brooke, M. H., Fenichel, G. M., Griggs, R. C., Mendell, J. R., Moxley, R., Miller, J. P. & Province, M. A. 1983. Clinical investigation in Duchenne dystrophy: 2. Determination of the "power" of therapeutic trials based on the natural history. *Muscle Nerve*, **6**, 91-103.
- Brooks, B. R. 1994. El Escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis. Subcommittee on Motor Neuron Diseases/Amyotrophic Lateral Sclerosis of the World Federation of Neurology Research Group on Neuromuscular Diseases and the El Escorial "Clinical limits of amyotrophic lateral sclerosis" workshop contributors. *J Neurol Sci*, **124 Suppl**, 96-107.
- Brooks, B. R., Miller, R. G., Swash, M. & Munsat, T. L. 1998. El Escorial Revisited: Revised Criteria for the Diagnosis of Amyotrophic Lateral Sclerosis. World Federation of Neurology Research Group on Motor Neuron Diseases. Available at: <http://www.wfnals.org/guidelines/1998elescorial/elescorial1998.htm>.
- Bruijn, L. I., Becher, M. W., Lee, M. K., Anderson, K. L., Jenkins, N. A., Copeland, N. G., Sisodia, S. S., Rothstein, J. D., Borchelt, D. R., Price, D. L. & Cleveland, D. W. 1997. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron*, **18**, 327-38.
- Buss, A., Sellhaus, B., Wolmsley, A., Noth, J., Schwab, M. E. & Brook, G. A. 2004. Expression pattern of NOGO-A protein in the human nervous system. *Acta Neuropathol (Berl)*.
- Busson, M., Carazo, A., Seyer, P., Grandemange, S., Casas, F., Pessemesse, L., Rouault, J. P., Wrutniak-Cabello, C. & Cabello, G. 2005. Coactivation of nuclear receptors and myogenic factors induces the major BTG1 influence on muscle differentiation. *Oncogene*, **24**, 1698-710.
- Cameron, A. & Rosenfeld, J. 2002. Nutritional issues and supplements in amyotrophic lateral sclerosis and other neurodegenerative disorders. *Curr Opin Clin Nutr Metab Care*, **5**, 631-43.
- Camu, W., Khoris, J., Moulard, B., Salachas, F., Briolotti, V., Rouleau, G. A. & Meininger, V. 1999. Genetics of familial ALS and consequences for diagnosis. French ALS Research Group. *J Neurol Sci*, **165 Suppl 1**, S21-6.
- Caroni, P. & Schwab, M. E. 1988. Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate properties of CNS white matter. *Neuron*, **1**, 85-96.
- Carri, M. T., Ferri, A., Battistoni, A., Famhy, L., Gabbianelli, R., Poccia, F. & Rotilio, G. 1997. Expression of a Cu,Zn superoxide dismutase typical of familial amyotrophic lateral sclerosis induces mitochondrial alteration and increase of cytosolic Ca²⁺ concentration in transfected neuroblastoma SH-SY5Y cells. *FEBS Lett*, **414**, 365-8.

- Cashman, N. R. 1999. Do the benefits of currently available treatments justify early diagnosis and announcement? Arguments for. *Neurology*, **53**, S50-2; discussion S55-7.
- Cedarbaum, J. M., Stambler, N., Malta, E., Fuller, C., Hilt, D., Thurmond, B. & Nakanishi, A. 1999. The ALSFRS-R: a revised ALS functional rating scale that incorporates assessments of respiratory function. BDNF ALS Study Group (Phase III). *J Neurol Sci*, **169**, 13-21.
- Charcot, J. M. 1874. De la sclérose latérale amyotrophique. *Prog Med*, **2**, 325-327, 341-342, 453-455.
- Chen, M. S., Huber, A. B., van der Haar, M. E., Frank, M., Schnell, L., Spillmann, A., Christ, F. & Schwab, M. E. 2000. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature*, **403**, 434-9.
- Chen, Y. Z., Bennett, C. L., Huynh, H. M., Blair, I. P., Puls, I., Irobi, J., Dierick, I., Abel, A., Kennerson, M. L., Rabin, B. A., Nicholson, G. A., Auer-Grumbach, M., Wagner, K., De Jonghe, P., Griffin, J. W., Fischbeck, K. H., Timmerman, V., Cornblath, D. R. & Chance, P. F. 2004. DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am J Hum Genet*, **74**, 1128-35.
- Cheng, J., Sun, S., Tracy, A., Hubbell, E., Morris, J., Valmeekam, V., Kimbrough, A., Cline, M. S., Liu, G., Shigeta, R., Kulp, D. & Siani-Rose, M. A. 2004. NetAffx Gene Ontology Mining Tool: a visual approach for microarray data analysis. *Bioinformatics*, **20**, 1462-3.
- Cheung, G., Gawel, M. J., Cooper, P. W., Farb, R. I., Ang, L. C. & Gawal, M. J. 1995. Amyotrophic lateral sclerosis: correlation of clinical and MR imaging findings. *Radiology*, **194**, 263-70.
- Chiu, A. Y., Zhai, P., Dal Canto, M. C., Peters, T. M., Kwon, Y. W., Prattis, S. M. & Gurney, M. E. 1995. Age-dependent penetrance of disease in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Mol Cell Neurosci*, **6**, 349-62.
- Chung, M. J. & Suh, Y. L. 2002. Ultrastructural changes of mitochondria in the skeletal muscle of patients with amyotrophic lateral sclerosis. *Ultrastruct Pathol*, **26**, 3-7.
- Clapham, J. C., Arch, J. R., Chapman, H., Haynes, A., Lister, C., Moore, G. B., Piercy, V., Carter, S. A., Lehner, I., Smith, S. A., Beeley, L. J., Godden, R. J., Herrity, N., Skehel, M., Changani, K. K., Hockings, P. D., Reid, D. G., Squires, S. M., Hatcher, J., Trail, B., Latcham, J., Rastan, S., Harper, A. J., Cadenas, S., Buckingham, J. A., Brand, M. D. & Abuin, A. 2000. Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature*, **406**, 415-8.

- Claus, D., Brunholz, C., Kerling, F. P. & Henschel, S. 1995. Transcranial magnetic stimulation as a diagnostic and prognostic test in amyotrophic lateral sclerosis. *J Neurol Sci*, **129 Suppl**, 30-4.
- Clement, A. M., Nguyen, M. D., Roberts, E. A., Garcia, M. L., Boillee, S., Rule, M., McMahon, A. P., Doucette, W., Siwek, D., Ferrante, R. J., Brown, R. H., Jr., Julien, J. P., Goldstein, L. S. & Cleveland, D. W. 2003. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science*, **302**, 113-7.
- Cline, G. W., Vidal-Puig, A. J., Dufour, S., Cadman, K. S., Lowell, B. B. & Shulman, G. I. 2001. In vivo effects of uncoupling protein-3 gene disruption on mitochondrial energy metabolism. *J Biol Chem*, **276**, 20240-4.
- Coleman, M. P. & Perry, V. H. 2002. Axon pathology in neurological disease: a neglected therapeutic target. *Trends Neurosci*, **25**, 532-7.
- Corcia, P., Mayeux-Portas, V., Khoris, J., de Toffol, B., Autret, A., Muh, J. P., Camu, W. & Andres, C. 2002. Abnormal SMN1 gene copy number is a susceptibility factor for amyotrophic lateral sclerosis. *Ann Neurol*, **51**, 243-6.
- Corcoran, J. & Maden, M. 1999. Nerve growth factor acts via retinoic acid synthesis to stimulate neurite outgrowth. *Nat Neurosci*, **2**, 307-8.
- Corcoran, J., So, P. L. & Maden, M. 2002. Absence of retinoids can induce motoneuron disease in the adult rat and a retinoid defect is present in motoneuron disease patients. *J Cell Sci*, **115**, 4735-41.
- Corti, S., Locatelli, F., Donadoni, C., Guglieri, M., Papadimitriou, D., Strazzer, S., Del Bo, R. & Comi, G. P. 2004. Wild-type bone marrow cells ameliorate the phenotype of SOD1-G93A ALS mice and contribute to CNS, heart and skeletal muscle tissues. *Brain*, **127**, 2518-32.
- Couillard-Despres, S., Zhu, Q., Wong, P. C., Price, D. L., Cleveland, D. W. & Julien, J. P. 1998. Protective effect of neurofilament heavy gene overexpression in motor neuron disease induced by mutant superoxide dismutase. *Proc Natl Acad Sci U S A*, **95**, 9626-30.
- Cwik, V. A., Hanstock, C. C., Allen, P. S. & Martin, W. R. 1998. Estimation of brainstem neuronal loss in amyotrophic lateral sclerosis with in vivo proton magnetic resonance spectroscopy. *Neurology*, **50**, 72-7.
- Dahlquist, K. D., Salomonis, N., Vranizan, K., Lawlor, S. C. & Conklin, B. R. 2002. GenMAPP, a new tool for viewing and analyzing microarray data on biological pathways. *Nat Genet*, **31**, 19-20.
- Dal Canto, M. C. & Gurney, M. E. 1995. Neuropathological changes in two lines of mice carrying a transgene for mutant human Cu,Zn SOD, and in mice overexpressing wild type human SOD: a model of familial amyotrophic lateral sclerosis (FALS). *Brain Res*, **676**, 25-40.

- Dal Canto, M. C. & Gurney, M. E. 1997. A low expressor line of transgenic mice carrying a mutant human Cu,Zn superoxide dismutase (SOD1) gene develops pathological changes that most closely resemble those in human amyotrophic lateral sclerosis. *Acta Neuropathol (Berl)*, **93**, 537-50.
- Dalakas, M. C., Hatazawa, J., Brooks, R. A. & Di Chiro, G. 1987. Lowered cerebral glucose utilization in amyotrophic lateral sclerosis. *Ann Neurol*, **22**, 580-6.
- Dantes, M. & McComas, A. 1991. The extent and time course of motoneuron involvement in amyotrophic lateral sclerosis. *Muscle Nerve*, **14**, 416-21.
- Das, N. P. 1989. Effects of vitamin A and its analogs on nonenzymatic lipid peroxidation in rat brain mitochondria. *J Neurochem*, **52**, 585-8.
- Deckwerth, T. L. & Johnson, E. M., Jr. 1994. Neurites can remain viable after destruction of the neuronal soma by programmed cell death (apoptosis). *Dev Biol*, **165**, 63-72.
- Demmig-Adams, B. & Adams, W. W., 3rd. 2002. Antioxidants in photosynthesis and human nutrition. *Science*, **298**, 2149-53.
- Deng, H. X., Hentati, A., Tainer, J. A., Iqbal, Z., Cayabyab, A., Hung, W. Y., Getzoff, E. D., Hu, P., Herzfeldt, B., Roos, R. P. & et al. 1993. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. *Science*, **261**, 1047-51.
- Desport, J. C., Preux, P. M., Magy, L., Boirie, Y., Vallat, J. M., Beaufriere, B. & Couratier, P. 2001. Factors correlated with hypermetabolism in patients with amyotrophic lateral sclerosis. *Am J Clin Nutr*, **74**, 328-34.
- Desport, J. C., Preux, P. M., Truong, T. C., Vallat, J. M., Sautereau, D. & Couratier, P. 1999. Nutritional status is a prognostic factor for survival in ALS patients. *Neurology*, **53**, 1059-63.
- D'Haeseleer, P., Liang, S. & Somogyi, R. 2000. Genetic network inference: from co-expression clustering to reverse engineering. *Bioinformatics*, **16**, 707-26.
- Di Sano, F., Fazi, B., Citro, G., Lovat, P. E., Cesareni, G. & Piacentini, M. 2003. Glucosylceramide synthase and its functional interaction with RTN-1C regulate chemotherapeutic-induced apoptosis in neuroepithelioma cells. *Cancer Res*, **63**, 3860-5.
- Dobrowolny, G., Giacinti, C., Pelosi, L., Nicoletti, C., Winn, N., Barberi, L., Molinaro, M., Rosenthal, N. & Musaro, A. 2005. Muscle expression of a local Igf-1 isoform protects motor neurons in an ALS mouse model. *J Cell Biol*, **168**, 193-9.
- Dodd, D. A., Niederoest, B., Bloechlinger, S., Dupuis, L., Loeffler, J. P. & Schwab, M. E. 2005. Nogo-A, -B, and -C are found on the cell surface and interact together in many different cell types. *J Biol Chem*, **280**, 12494-502.

- Dugan, L. L., Turetsky, D. M., Du, C., Lobner, D., Wheeler, M., Almlı, C. R., Shen, C. K., Luh, T. Y., Choi, D. W. & Lin, T. S. 1997. Carboxyfullerenes as neuroprotective agents. *Proc Natl Acad Sci U S A*, **94**, 9434-9.
- Dupuis, L., Oudart, H., Rene, F., Gonzalez de Aguilar, J. L. & Loeffler, J. P. 2004. Evidence for defective energy homeostasis in amyotrophic lateral sclerosis: benefit of a high-energy diet in a transgenic mouse model. *Proc Natl Acad Sci U S A*, **101**, 11159-64.
- Echtay, K. S., Roussel, D., St-Pierre, J., Jekabsons, M. B., Cadenas, S., Stuart, J. A., Harper, J. A., Roebuck, S. J., Morrison, A., Pickering, S., Clapham, J. C. & Brand, M. D. 2002. Superoxide activates mitochondrial uncoupling proteins. *Nature*, **415**, 96-9.
- Eisen, A., Entezari-Taher, M. & Stewart, H. 1996. Cortical projections to spinal motoneurons: changes with aging and amyotrophic lateral sclerosis. *Neurology*, **46**, 1396-404.
- Eisen, M. B., Spellman, P. T., Brown, P. O. & Botstein, D. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci U S A*, **95**, 14863-8.
- Emerick, A. J., Neafsey, E. J., Schwab, M. E. & Kartje, G. L. 2003. Functional reorganization of the motor cortex in adult rats after cortical lesion and treatment with monoclonal antibody IN-1. *J Neurosci*, **23**, 4826-30.
- Fahn, S. & Elton, E. R. 1987. Unified Parkinson's Disease Rating Scale. In: Fahn S, Marsden CD, Calne D, Goldstein M, eds. *Recent Developments in Parkinson's Disease*, Florham Park, NJ: Macmillan Press, 293-304.
- Felipe, F., Bonet, M. L., Ribot, J. & Palou, A. 2003. Up-regulation of muscle uncoupling protein 3 gene expression in mice following high fat diet, dietary vitamin A supplementation and acute retinoic acid-treatment. *Int J Obes Relat Metab Disord*, **27**, 60-9.
- Figlewicz, D. A., Garruto, R. M., Krizus, A., Yanagihara, R. & Rouleau, G. A. 1994. The Cu/Zn superoxide dismutase gene in ALS and parkinsonism-dementia of Guam. *Neuroreport*, **5**, 557-60.
- Finn, J. T., Weil, M., Archer, F., Siman, R., Srinivasan, A. & Raff, M. C. 2000. Evidence that Wallerian degeneration and localized axon degeneration induced by local neurotrophin deprivation do not involve caspases. *J Neurosci*, **20**, 1333-41.
- Fischer, L. R., Culver, D. G., Tennant, P., Davis, A. A., Wang, M., Castellano-Sanchez, A., Khan, J., Polak, M. A. & Glass, J. D. 2004. Amyotrophic lateral sclerosis is a distal axonopathy: evidence in mice and man. *Exp Neurol*, **185**, 232-40.
- Flandin, P., Donati, Y., Barazzone-Argiroffo, C. & Muzzin, P. 2005. Hyperoxia-mediated oxidative stress increases expression of UCP3 mRNA and protein in skeletal muscle. *FEBS Lett*, **579**, 3411-5.

- Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D. & Warden, C. H. 1997. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat Genet*, **15**, 269-72.
- Fontoura, P., Ho, P. P., DeVoss, J., Zheng, B., Lee, B. J., Kidd, B. A., Garren, H., Sobel, R. A., Robinson, W. H., Tessier-Lavigne, M. & Steinman, L. 2004. Immunity to the extracellular domain of Nogo-A modulates experimental autoimmune encephalomyelitis. *J Immunol*, **173**, 6981-92.
- Fournier, A. E., GrandPre, T. & Strittmatter, S. M. 2001. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. *Nature*, **409**, 341-6.
- Frey, D., Schneider, C., Xu, L., Borg, J., Spooren, W. & Caroni, P. 2000. Early and selective loss of neuromuscular synapse subtypes with low sprouting competence in motoneuron diseases. *J Neurosci*, **20**, 2534-42.
- Friedlander, R. M., Brown, R. H., Gagliardini, V., Wang, J. & Yuan, J. 1997. Inhibition of ICE slows ALS in mice. *Nature*, **388**, 31.
- Funakoshi, H., Belluardo, N., Arenas, E., Yamamoto, Y., Casabona, A., Persson, H. & Ibanez, C. F. 1995. Muscle-derived neurotrophin-4 as an activity-dependent trophic signal for adult motor neurons. *Science*, **268**, 1495-9.
- Gajdusek, D. C. & Salazar, A. M. 1982. Amyotrophic lateral sclerosis and parkinsonian syndromes in high incidence among the Auyu and Jakai people of West New Guinea. *Neurology*, **32**, 107-26.
- Gardner, D. G. & Chen, S. 1999. Retinoids and cell growth in the cardiovascular system. *Life Sci*, **65**, 1607-13.
- Garruto, R. M., Yanagihara, R. & Gajdusek, D. C. 1985. Disappearance of high-incidence amyotrophic lateral sclerosis and parkinsonism-dementia on Guam. *Neurology*, **35**, 193-8.
- Geisler, J. G., Palmer, R. J., Stubbs, L. J. & Mucenski, M. L. 1999. Nspl1, a new Z-band-associated protein. *J Muscle Res Cell Motil*, **20**, 661-8.
- Georgiades, P. & Brickell, P. M. 1997. Differential expression of the rat retinoid X receptor gamma gene during skeletal muscle differentiation suggests a role in myogenesis. *Dev Dyn*, **210**, 227-35.
- Getz, G., Levine, E. & Domany, E. 2000. Coupled two-way clustering analysis of gene microarray data. *Proc Natl Acad Sci U S A*, **97**, 12079-84.
- Gimeno, R. E., Dembski, M., Weng, X., Deng, N., Shyjan, A. W., Gimeno, C. J., Iris, F., Ellis, S. J., Woolf, E. A. & Tartaglia, L. A. 1997. Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. *Diabetes*, **46**, 900-6.
- Glass, J. D. 2004. Wallerian degeneration as a window to peripheral neuropathy. *J Neurol Sci*, **220**, 123-4.

- Godoy, C., Arellano, M., Diaz, M., Duran, A. & Perez, P. 1996. Characterization of cwl1+, a gene from *Schizosaccharomyces pombe* whose overexpression causes cell lysis. *Yeast*, **12**, 983-90.
- Goglia, F. & Skulachev, V. P. 2003. A function for novel uncoupling proteins: antioxidant defense of mitochondrial matrix by translocating fatty acid peroxides from the inner to the outer membrane leaflet. *Faseb J*, **17**, 1585-91.
- Gong, D. W., He, Y., Karas, M. & Reitman, M. 1997. Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. *J Biol Chem*, **272**, 24129-32.
- Gong, Y. H., Parsadanian, A. S., Andreeva, A., Snider, W. D. & Elliott, J. L. 2000. Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. *J Neurosci*, **20**, 660-5.
- Gonzalez de Aguilar, J. L., Dupuis, L., Oudart, H. & Loeffler, J. P. 2005. The metabolic hypothesis in amyotrophic lateral sclerosis: insights from mutant Cu/Zn-superoxide dismutase mice. *Biomed Pharmacother*, **59**, 190-6.
- GrandPre, T., Li, S. & Strittmatter, S. M. 2002. Nogo-66 receptor antagonist peptide promotes axonal regeneration. *Nature*, **417**, 547-51.
- GrandPre, T., Nakamura, F., Vartanian, T. & Strittmatter, S. M. 2000. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. *Nature*, **403**, 439-44.
- Great Lakes ALS Study Group. 2003. A comparison of muscle strength testing techniques in amyotrophic lateral sclerosis. *Neurology*, **61**, 1503-7.
- Groeneveld, G. J., Veldink, J. H., van der Tweel, I., Kalmijn, S., Beijer, C., de Visser, M., Wokke, J. H., Franssen, H. & van den Berg, L. H. 2003. A randomized sequential trial of creatine in amyotrophic lateral sclerosis. *Ann Neurol*, **53**, 437-45.
- Grohme, K., Maravic, M. V., Gasser, T. & Borasio, G. D. 2001. A case of amyotrophic lateral sclerosis with a very slow progression over 44 years. *Neuromuscul Disord*, **11**, 414-6.
- Gurney, M. E., Cutting, F. B., Zhai, P., Doble, A., Taylor, C. P., Andrus, P. K. & Hall, E. D. 1996. Benefit of vitamin E, riluzole, and gabapentin in a transgenic model of familial amyotrophic lateral sclerosis. *Ann Neurol*, **39**, 147-57.
- Gurney, M. E., Pu, H., Chiu, A. Y., Dal Canto, M. C., Polchow, C. Y., Alexander, D. D., Caliendo, J., Hentati, A., Kwon, Y. W., Deng, H. X. & et al. 1994. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science*, **264**, 1772-5.
- Haase, G., Kennel, P., Pettmann, B., Vigne, E., Akli, S., Revah, F., Schmalbruch, H. & Kahn, A. 1997. Gene therapy of murine motor neuron disease using adenoviral vectors for neurotrophic factors. *Nat Med*, **3**, 429-36.

- Hadano, S., Yanagisawa, Y., Skaug, J., Fichter, K., Nasir, J., Martindale, D., Koop, B. F., Scherer, S. W., Nicholson, D. W., Rouleau, G. A., Ikeda, J. & Hayden, M. R. 2001. Cloning and characterization of three novel genes, ALS2CR1, ALS2CR2, and ALS2CR3, in the juvenile amyotrophic lateral sclerosis (ALS2) critical region at chromosome 2q33-q34: candidate genes for ALS2. *Genomics*, **71**, 200-13.
- Hamada, N., Iwahashi, J., Suzuki, K., Ogi, H., Kashiwagi, T., Hara, K., Toyoda, M., Yamada, T. & Toyoda, T. 2002. Molecular cloning and characterization of the mouse reticulon 3 cDNA. *Cell Mol Biol (Noisy-le-grand)*, **48**, 163-72.
- Hatazawa, J., Brooks, R. A., Dalakas, M. C., Mansi, L. & Di Chiro, G. 1988. Cortical motor-sensory hypometabolism in amyotrophic lateral sclerosis: a PET study. *J Comput Assist Tomogr*, **12**, 630-6.
- Hauben, E., Ibarra, A., Mizrahi, T., Barouch, R., Agranov, E. & Schwartz, M. 2001. Vaccination with a Nogo-A-derived peptide after incomplete spinal-cord injury promotes recovery via a T-cell-mediated neuroprotective response: comparison with other myelin antigens. *Proc Natl Acad Sci U S A*, **98**, 15173-8.
- He, W., Lu, Y., Qahwash, I., Hu, X. Y., Chang, A. & Yan, R. 2004. Reticulon family members modulate BACE1 activity and amyloid-beta peptide generation. *Nat Med*, **10**, 959-65.
- Hens, J., Nuydens, R., Geerts, H., Senden, N. H., Van de Ven, W. J., Roebroek, A. J., van de Velde, H. J., Ramaekers, F. C. & Broers, J. L. 1998. Neuronal differentiation is accompanied by NSP-C expression. *Cell Tissue Res*, **292**, 229-37.
- Hillel, A. D., Miller, R. M., Yorkston, K., McDonald, E., Norris, F. H. & Konikow, N. 1989. Amyotrophic lateral sclerosis severity scale. *Neuroepidemiology*, **8**, 142-50.
- Hoagland, R. J., Mendoza, M., Armon, C., Barohn, R. J., Bryan, W. W., Goodpasture, J. C., Miller, R. G., Parry, G. J., Petajan, J. H. & Ross, M. A. 1997. Reliability of maximal voluntary isometric contraction testing in a multicenter study of patients with amyotrophic lateral sclerosis. Syntex/Synergen Neuroscience Joint Venture rhCNTF ALS Study Group. *Muscle Nerve*, **20**, 691-5.
- Houi, K., Kobayashi, T., Kato, S., Mochio, S. & Inoue, K. 2002. Increased plasma TGF-beta1 in patients with amyotrophic lateral sclerosis. *Acta Neurol Scand*, **106**, 299-301.
- Househam, E. & Swash, M. 2000. Diagnostic delay in amyotrophic lateral sclerosis: what scope for improvement? *J Neurol Sci*, **180**, 76-81.
- Hu, W. H., Hausmann, O. N., Yan, M. S., Walters, W. M., Wong, P. K. & Bethea, J. R. 2002. Identification and characterization of a novel Nogo-interacting mitochondrial protein (NIMP). *J Neurochem*, **81**, 36-45.

- Huber, A. B., Weinmann, O., Brosamle, C., Oertle, T. & Schwab, M. E. 2002. Patterns of Nogo mRNA and protein expression in the developing and adult rat and after CNS lesions. *J Neurosci*, **22**, 3553-67.
- Huddleston, O. L., Golseth, J. G., Marinacci, A. A. & Austin, E. 1950. The use of electromyography in the diagnosis of neuromuscular disorders. *Arch Phys Med Rehabil*, **31**, 378-87.
- Hughes, J. S., Lienhardt, H. F. & Aubel, C. E. 1924. Nerve degeneration resulting from avitaminosis A. *J Nutrition*, **2**, 183-186.
- Ihaka, R. & Gentleman, R. 1996. R: A language for data analysis and graphics. *J Comp Graph Stat*, **5**, 299-314.
- Ilzecka, J. 2003. Prostaglandin E2 is increased in amyotrophic lateral sclerosis patients. *Acta Neurol Scand*, **108**, 125-9.
- Ilzecka, J., Kocki, T., Stelmasiak, Z. & Turski, W. A. 2003. Endogenous protectant kynurenic acid in amyotrophic lateral sclerosis. *Acta Neurol Scand*, **107**, 412-8.
- Ilzecka, J. & Stelmasiak, Z. 2003a. Creatine kinase activity in amyotrophic lateral sclerosis patients. *Neurol Sci*, **24**, 286-7.
- Ilzecka, J. & Stelmasiak, Z. 2003b. Serum bilirubin concentration in patients with amyotrophic lateral sclerosis. *Clin Neurol Neurosurg*, **105**, 237-40.
- Ilzecka, J., Stelmasiak, Z. & Dobosz, B. 2001. Interleukin-1beta converting enzyme/Caspase-1 (ICE/Caspase-1) and soluble APO-1/Fas/CD 95 receptor in amyotrophic lateral sclerosis patients. *Acta Neurol Scand*, **103**, 255-8.
- Irizarry, R. A., Hobbs, B., Collin, F., Beazer-Barclay, Y. D., Antonellis, K. J., Scherf, U. & Speed, T. P. 2003. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics*, **4**, 249-64.
- Irving, J. T. & Richards, M. B. 1938. Early lesions of vitamin A deficiency. *J Physiol*, **94**, 307-321.
- Iwahashi, J. & Hamada, N. 2003. Human reticulon 1-A and 1-B interact with a medium chain of the AP-2 adaptor complex. *Cell Mol Biol (Noisy-le-grand)*, **49** **Online Pub**, OL467-71.
- Iwahashi, J., Kawasaki, I., Kohara, Y., Gengyo-Ando, K., Mitani, S., Ohshima, Y., Hamada, N., Hara, K., Kashiwagi, T. & Toyoda, T. 2002. Caenorhabditis elegans reticulon interacts with RME-1 during embryogenesis. *Biochem Biophys Res Commun*, **293**, 698-704.
- Iwasaki, Y., Ikeda, K. & Kinoshita, M. 1995. Vitamin A and E levels are normal in amyotrophic lateral sclerosis. *J Neurol Sci*, **132**, 193-4.
- Iwasaki, Y., Ikeda, K., Shiojima, T. & Kinoshita, M. 1992. Increased plasma concentrations of aspartate, glutamate and glycine in Parkinson's disease. *Neurosci Lett*, **145**, 175-7.

- Jaburek, M., Miyamoto, S., Di Mascio, P., Garlid, K. D. & Jezek, P. 2004. Hydroperoxy fatty acid cycling mediated by mitochondrial uncoupling protein UCP2. *J Biol Chem*, **279**, 53097-102.
- Jackson, M., Morrison, K. E., Al-Chalabi, A., Bakker, M. & Leigh, P. N. 1996. Analysis of chromosome 5q13 genes in amyotrophic lateral sclerosis: homozygous NAIP deletion in a sporadic case. *Ann Neurol*, **39**, 796-800.
- Jago, R. T., Lecker, S. H., Gomes, M. & Goldberg, A. L. 2002. Patterns of gene expression in atrophying skeletal muscles: response to food deprivation. *Faseb J*, **16**, 1697-712.
- Jezek, P., Engstova, H., Zackova, M., Vercesi, A. E., Costa, A. D., Arruda, P. & Garlid, K. D. 1998. Fatty acid cycling mechanism and mitochondrial uncoupling proteins. *Biochim Biophys Acta*, **1365**, 319-27.
- Johnston, M., Earll, L., Mitchell, E., Morrison, V. & Wright, S. 1996. Communicating the diagnosis of motor neurone disease. *Palliat Med*, **10**, 23-34.
- Jokic, N., Gonzalez de Aguilar, J. L., Pradat, P. F., Dupuis, L., Echaniz-Laguna, A., Muller, A., Dubourg, O., Seilhean, D., Hauw, J. J., Loeffler, J. P. & Meininger, V. 2005. Nogo expression in muscle correlates with amyotrophic lateral sclerosis severity. *Ann Neurol*, **57**, 553-6.
- Josephson, A., Widenfalk, J., Widmer, H. W., Olson, L. & Spenger, C. 2001. NOGO mRNA expression in adult and fetal human and rat nervous tissue and in weight drop injury. *Exp Neurol*, **169**, 319-28.
- Juergens, S. M., Kurland, L. T., Okazaki, H. & Mulder, D. W. 1980. ALS in Rochester, Minnesota, 1925-1977. *Neurology*, **30**, 463-70.
- Karnezis, T., Mandemakers, W., McQualter, J. L., Zheng, B., Ho, P. P., Jordan, K. A., Murray, B. M., Barres, B., Tessier-Lavigne, M. & Bernard, C. C. 2004. The neurite outgrowth inhibitor Nogo A is involved in autoimmune-mediated demyelination. *Nat Neurosci*, **7**, 736-44.
- Kasarskis, E. J., Berryman, S., Vanderleest, J. G., Schneider, A. R. & McClain, C. J. 1996. Nutritional status of patients with amyotrophic lateral sclerosis: relation to the proximity of death. *Am J Clin Nutr*, **63**, 130-7.
- Kaspar, B. K., Llado, J., Sherkat, N., Rothstein, J. D. & Gage, F. H. 2003. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science*, **301**, 839-42.
- Kaye, J. A. 2000. Methods for discerning disease-modifying effects in Alzheimer disease treatment trials. *Arch Neurol*, **57**, 312-4.
- Kennel, P. F., Finiels, F., Revah, F. & Mallet, J. 1996. Neuromuscular function impairment is not caused by motor neurone loss in FALS mice: an electromyographic study. *Neuroreport*, **7**, 1427-31.

- Kerner, J., Turkaly, P. J., Minkler, P. E. & Hoppel, C. L. 2001. Aging skeletal muscle mitochondria in the rat: decreased uncoupling protein-3 content. *Am J Physiol Endocrinol Metab*, **281**, E1054-62.
- Kew, J. J., Brooks, D. J., Passingham, R. E., Rothwell, J. C., Frackowiak, R. S. & Leigh, P. N. 1994. Cortical function in progressive lower motor neuron disorders and amyotrophic lateral sclerosis: a comparative PET study. *Neurology*, **44**, 1101-10.
- Kim, J. E., Li, S., GrandPre, T., Qiu, D. & Strittmatter, S. M. 2003. Axon regeneration in young adult mice lacking Nogo-A/B. *Neuron*, **38**, 187-99.
- Kim, J. E., Liu, B. P., Park, J. H. & Strittmatter, S. M. 2004. Nogo-66 receptor prevents raphespinal and rubrospinal axon regeneration and limits functional recovery from spinal cord injury. *Neuron*, **44**, 439-51.
- Kim, S. H., Yoo, B. C., Broers, J. L., Cairns, N. & Lubec, G. 2000. Neuroendocrine-specific protein C, a marker of neuronal differentiation, is reduced in brain of patients with Down syndrome and Alzheimer's disease. *Biochem Biophys Res Commun*, **276**, 329-34.
- Kitamura, M., Ishikawa, Y., Moreno-Manzano, V., Xu, Q., Konta, T., Lucio-Cazana, J., Furusu, A. & Nakayama, K. 2002. Intervention by retinoic acid in oxidative stress-induced apoptosis. *Nephrol Dial Transplant*, **17 Suppl 9**, 84-7.
- Klingenberg, M., Echtay, K. S., Bienengraeber, M., Winkler, E. & Huang, S. G. 1999. Structure-function relationship in UCP1. *Int J Obes Relat Metab Disord*, **23 Suppl 6**, S24-9.
- Klivenyi, P., Ferrante, R. J., Matthews, R. T., Bogdanov, M. B., Klein, A. M., Andreassen, O. A., Mueller, G., Wermer, M., Kaddurah-Daouk, R. & Beal, M. F. 1999. Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat Med*, **5**, 347-50.
- Kong, J. & Xu, Z. 1998. Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *J Neurosci*, **18**, 3241-50.
- Kools, P. F., Roebroek, A. J., Van de Velde, H. J., Marynen, P., Bullerdiek, J. & Van de Ven, W. J. 1994. Regional mapping of the human NSP gene to chromosome region 14q21-->q22 by fluorescence in situ hybridization analysis. *Cytogenet Cell Genet*, **66**, 48-50.
- Kostrominova, T. Y., Dow, D. E., Dennis, R. G., Miller, R. A. & Faulkner, J. A. 2005. Comparison of gene expression of 2-mo denervated, 2-mo stimulated-denervated, and control rat skeletal muscles. *Physiol Genomics*, **22**, 227-43.
- Kumamaru, E., Kuo, C. H., Fujimoto, T., Kohama, K., Zeng, L. H., Taira, E., Tanaka, H., Toyoda, T. & Miki, N. 2004. Reticulon3 expression in rat optic and olfactory systems. *Neurosci Lett*, **356**, 17-20.

- Kusui, K. 1962. [Epidemiological study on amyotrophic lateral sclerosis (ALS) and other neighboring motor neuron diseases in Kii Peninsula.]. *Folia Psychiatr Neurol Jpn*, **64**, 85-99.
- Lacomblez, L., Bensimon, G., Leigh, P. N., Guillet, P. & Meininger, V. 1996. Dose-ranging study of riluzole in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis/Riluzole Study Group II. *Lancet*, **347**, 1425-31.
- Lai, E. C., Felice, K. J., Festoff, B. W., Gawel, M. J., Gelinias, D. F., Kratz, R., Murphy, M. F., Natter, H. M., Norris, F. H. & Rudnicki, S. A. 1997. Effect of recombinant human insulin-like growth factor-I on progression of ALS. A placebo-controlled study. The North America ALS/IGF-I Study Group. *Neurology*, **49**, 1621-30.
- Lambert, E. H. & Mulder, D. W. 1957. Electromyographic studies in amyotrophic lateral sclerosis. *Mayo Clin Proc*, **32**, 441-6.
- Larrain, J., Cizmeci-Smith, G., Troncoso, V., Stahl, R. C., Carey, D. J. & Brandan, E. 1997. Syndecan-1 expression is down-regulated during myoblast terminal differentiation. Modulation by growth factors and retinoic acid. *J Biol Chem*, **272**, 18418-24.
- Lecker, S. H., Jagoe, R. T., Gilbert, A., Gomes, M., Baracos, V., Bailey, J., Price, S. R., Mitch, W. E. & Goldberg, A. L. 2004. Multiple types of skeletal muscle atrophy involve a common program of changes in gene expression. *Faseb J*, **18**, 39-51.
- Leclerc, N., Ribera, F., Zoll, J., Warter, J. M., Poindron, P., Lampert, E. & Borg, J. 2001. Selective changes in mitochondria respiratory properties in oxidative or glycolytic muscle fibers isolated from G93AhumanSOD1 transgenic mice. *Neuromuscul Disord*, **11**, 722-7.
- Lee, J. K., Kim, J. E., Sivula, M. & Strittmatter, S. M. 2004. Nogo receptor antagonism promotes stroke recovery by enhancing axonal plasticity. *J Neurosci*, **24**, 6209-17.
- Li, Q., Li, Z., Sun, C. X. & Yu, A. C. 2002. Identification of transcripts expressed under functional differentiation in primary culture of cerebral cortical neurons. *Neurochem Res*, **27**, 147-54.
- Li, Q., Qi, B., Oka, K., Shimakage, M., Yoshioka, N., Inoue, H., Hakura, A., Kodama, K., Stanbridge, E. J. & Yutsudo, M. 2001. Link of a new type of apoptosis-inducing gene ASY/Nogo-B to human cancer. *Oncogene*, **20**, 3929-36.
- Li, S., Liu, B. P., Budel, S., Li, M., Ji, B., Walus, L., Li, W., Jirik, A., Rabacchi, S., Choi, E., Worley, D., Sah, D. W., Pepinsky, B., Lee, D., Relton, J. & Strittmatter, S. M. 2004. Blockade of Nogo-66, myelin-associated glycoprotein, and oligodendrocyte myelin glycoprotein by soluble Nogo-66 receptor promotes axonal sprouting and recovery after spinal injury. *J Neurosci*, **24**, 10511-20.
- Li, T. M., Alberman, E. & Swash, M. 1990. Clinical features and associations of 560 cases of motor neuron disease. *J Neurol Neurosurg Psychiatry*, **53**, 1043-5.

- Lilienfeld, D. E., Chan, E., Ehland, J., Godbold, J., Landrigan, P. J., Marsh, G. & Perl, D. P. 1989. Rising mortality from motoneuron disease in the USA, 1962-84. *Lancet*, **1**, 710-3.
- Lin, C. L., Bristol, L. A., Jin, L., Dykes-Hoberg, M., Crawford, T., Clawson, L. & Rothstein, J. D. 1998. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. *Neuron*, **20**, 589-602.
- Lino, M. M., Schneider, C. & Caroni, P. 2002. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J Neurosci*, **22**, 4825-32.
- Liochev, S. I. & Fridovich, I. 2003. Mutant Cu,Zn superoxide dismutases and familial amyotrophic lateral sclerosis: evaluation of oxidative hypotheses. *Free Radic Biol Med*, **34**, 1383-9.
- Lipshutz, R. J., Fodor, S. P., Gingeras, T. R. & Lockhart, D. J. 1999. High density synthetic oligonucleotide arrays. *Nat Genet*, **21**, 20-4.
- Liu, G., Loraine, A. E., Shigeta, R., Cline, M., Cheng, J., Valmeekam, V., Sun, S., Kulp, D. & Siani-Rose, M. A. 2003. NetAffx: Affymetrix probesets and annotations. *Nucleic Acids Res*, **31**, 82-6.
- Lloyd, C. M., Richardson, M. P., Brooks, D. J., Al-Chalabi, A. & Leigh, P. N. 2000. Extramotor involvement in ALS: PET studies with the GABA(A) ligand [(11)C]flumazenil. *Brain*, **123** (Pt 11), 2289-96.
- Lock, C., Hermans, G., Pedotti, R., Brendolan, A., Schadt, E., Garren, H., Langer-Gould, A., Strober, S., Cannella, B., Allard, J., Klonowski, P., Austin, A., Lad, N., Kaminski, N., Galli, S. J., Oksenberg, J. R., Raine, C. S., Heller, R. & Steinman, L. 2002. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat Med*, **8**, 500-8.
- Lockhart, D. J., Dong, H., Byrne, M. C., Follettie, M. T., Gallo, M. V., Chee, M. S., Mittmann, M., Wang, C., Kobayashi, M., Horton, H. & Brown, E. L. 1996. Expression monitoring by hybridization to high-density oligonucleotide arrays. *Nat Biotechnol*, **14**, 1675-80.
- Lu, Y. Y., Wang, L. J., Muramatsu, S., Ikeguchi, K., Fujimoto, K., Okada, T., Mizukami, H., Matsushita, T., Hanazono, Y., Kume, A., Nagatsu, T., Ozawa, K. & Nakano, I. 2003. Intramuscular injection of AAV-GDNF results in sustained expression of transgenic GDNF, and its delivery to spinal motoneurons by retrograde transport. *Neurosci Res*, **45**, 33-40.
- Ludolph, A. C. & Riepe, M. W. 1999. Do the benefits of currently available treatments justify early diagnosis and treatment of amyotrophic lateral sclerosis? Arguments against. *Neurology*, **53**, S46-9; discussion S55-7.
- Maclellan, J. D., Gerrits, M. F., Gowing, A., Smith, P. J., Wheeler, M. B. & Harper, M. E. 2005. Physiological increases in uncoupling protein 3 augment Fatty Acid

- oxidation and decrease reactive oxygen species production without uncoupling respiration in muscle cells. *Diabetes*, **54**, 2343-50.
- Maden, M. & Hind, M. 2003. Retinoic acid, a regeneration-inducing molecule. *Dev Dyn*, **226**, 237-44.
- Magnusson, C., Libelius, R. & Tagerud, S. 2003. Nogo (Reticulon 4) expression in innervated and denervated mouse skeletal muscle. *Mol Cell Neurosci*, **22**, 298-307.
- Malaspina, A., Kaushik, N. & de Bellerocche, J. 2001. Differential expression of 14 genes in amyotrophic lateral sclerosis spinal cord detected using gridded cDNA arrays. *J Neurochem*, **77**, 132-45.
- Manabe, Y., Nagano, I., Gazi, M. S., Murakami, T., Shiote, M., Shoji, M., Kitagawa, H., Setoguchi, Y. & Abe, K. 2002. Adenovirus-mediated gene transfer of glial cell line-derived neurotrophic factor prevents motor neuron loss of transgenic model mice for amyotrophic lateral sclerosis. *Apoptosis*, **7**, 329-34.
- Marinacci, A. 1955. Clinical Electromyography. *Los Angeles: San Lucas Press*.
- Maselli, R. A., Wollman, R. L., Leung, C., Distad, B., Palombi, S., Richman, D. P., Salazar-Grueso, E. F. & Roos, R. P. 1993. Neuromuscular transmission in amyotrophic lateral sclerosis. *Muscle Nerve*, **16**, 1193-203.
- Masu, Y., Wolf, E., Holtmann, B., Sendtner, M., Brem, G. & Thoenen, H. 1993. Disruption of the CNTF gene results in motor neuron degeneration. *Nature*, **365**, 27-32.
- McKusick, V. A. 1998. Mendelian Inheritance in Man. *Baltimore: Johns Hopkins University Press*, **12th ed.**
- Medical Research Council. 1976. Aids to the examination of the peripheral nervous system. *London: Her Majesty's Stationery Office*, **14**.
- Meier, S., Brauer, A. U., Heimrich, B., Schwab, M. E., Nitsch, R. & Savaskan, N. E. 2003. Molecular analysis of Nogo expression in the hippocampus during development and following lesion and seizure. *Faseb J*, **17**, 1153-5.
- Merkler, D., Metz, G. A., Raineteau, O., Dietz, V., Schwab, M. E. & Fouad, K. 2001. Locomotor recovery in spinal cord-injured rats treated with an antibody neutralizing the myelin-associated neurite growth inhibitor Nogo-A. *J Neurosci*, **21**, 3665-73.
- Merkler, D., Oertle, T., Buss, A., Pinschewer, D. D., Schnell, L., Bareyre, F. M., Kerschensteiner, M., Buddeberg, B. S. & Schwab, M. E. 2003. Rapid induction of autoantibodies against Nogo-A and MOG in the absence of an encephalitogenic T cell response: implication for immunotherapeutic approaches in neurological diseases. *Faseb J*, **17**, 2275-7.
- Meyer, T., Alber, B., Roemer, K., Martin, T., Kalscheuer, V. M., Gottert, E., Zang, K. D., Ludolph, A. C., Ropers, H. H. & Prudlo, J. 2003. High rate of constitutional

- chromosomal rearrangements in apparently sporadic ALS. *Neurology*, **60**, 1348-50.
- Mi, S., Lee, X., Shao, Z., Thill, G., Ji, B., Relton, J., Levesque, M., Allaire, N., Perrin, S., Sands, B., Crowell, T., Cate, R. L., McCoy, J. M. & Pepinsky, R. B. 2004. LINGO-1 is a component of the Nogo-66 receptor/p75 signaling complex. *Nat Neurosci*, **7**, 221-8.
- Miano, J. M. & Berk, B. C. 2000. Retinoids: versatile biological response modifiers of vascular smooth muscle phenotype. *Circ Res*, **87**, 355-62.
- Mic, F. A. & Duester, G. 2003. Patterning of forelimb bud myogenic precursor cells requires retinoic acid signaling initiated by Raldh2. *Dev Biol*, **264**, 191-201.
- Michel, A., Fischer, U. & Meese, E. 1999. A reticulon gene localized on chromosome 2 encodes a protein with increased autoimmunogenic properties in pilocytic astrocytoma patients. *Electron J Pathol Histol*, **5**, 993-997.
- Miller, R. G., Munsat, T. L., Swash, M. & Brooks, B. R. 1999a. Consensus guidelines for the design and implementation of clinical trials in ALS. World Federation of Neurology committee on Research. *J Neurol Sci*, **169**, 2-12.
- Miller, R. G., Rosenberg, J. A., Gelinas, D. F., Mitsumoto, H., Newman, D., Sufit, R., Borasio, G. D., Bradley, W. G., Bromberg, M. B., Brooks, B. R., Kasarskis, E. J., Munsat, T. L. & Oppenheimer, E. A. 1999b. Practice parameter: the care of the patient with amyotrophic lateral sclerosis (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology: ALS Practice Parameters Task Force. *Neurology*, **52**, 1311-23.
- Mingorance, A., Fontana, X., Sole, M., Burgaya, F., Urena, J. M., Teng, F. Y., Tang, B. L., Hunt, D., Anderson, P. N., Bethea, J. R., Schwab, M. E., Soriano, E. & del Rio, J. A. 2004. Regulation of Nogo and Nogo receptor during the development of the entorhino-hippocampal pathway and after adult hippocampal lesions. *Mol Cell Neurosci*, **26**, 34-49.
- Mirowitz, S., Sartor, K., Gado, M. & Torack, R. 1989. Focal signal-intensity variations in the posterior internal capsule: normal MR findings and distinction from pathologic findings. *Radiology*, **172**, 535-9.
- Mohajeri, M. H., Figlewicz, D. A. & Bohn, M. C. 1999. Intramuscular grafts of myoblasts genetically modified to secrete glial cell line-derived neurotrophic factor prevent motoneuron loss and disease progression in a mouse model of familial amyotrophic lateral sclerosis. *Hum Gene Ther*, **10**, 1853-66.
- Molina, J. A., de Bustos, F., Jimenez-Jimenez, F. J., Esteban, J., Guerrero-Sola, A., Zurdo, M., Orti-Pareja, M., Gasalla, T., Gomez-Escalonilla, C., Ramirez-Ramos, C., Guillamon, F. & Arenas, J. 1999. Serum levels of beta-carotene, alpha-carotene, and vitamin A in patients with amyotrophic lateral sclerosis. *Acta Neurol Scand*, **99**, 315-7.

- Moll, J., Barzaghi, P., Lin, S., Bezakova, G., Lochmuller, H., Engvall, E., Muller, U. & Ruegg, M. A. 2001. An agrin minigene rescues dystrophic symptoms in a mouse model for congenital muscular dystrophy. *Nature*, **413**, 302-7.
- Moreira, E. F., Jaworski, C. J. & Rodriguez, I. R. 1999. Cloning of a novel member of the reticulon gene family (RTN3): gene structure and chromosomal localization to 11q13. *Genomics*, **58**, 73-81.
- Moulard, B., Salachas, F., Chassande, B., Briolotti, V., Meininger, V., Malafosse, A. & Camu, W. 1998. Association between centromeric deletions of the SMN gene and sporadic adult-onset lower motor neuron disease. *Ann Neurol*, **43**, 640-4.
- Moulard, B., Sefiani, A., Laamri, A., Malafosse, A. & Camu, W. 1996. Apolipoprotein E genotyping in sporadic amyotrophic lateral sclerosis: evidence for a major influence on the clinical presentation and prognosis. *J Neurol Sci*, **139 Suppl**, 34-7.
- Mui, S., Rebeck, G. W., McKenna-Yasek, D., Hyman, B. T. & Brown, R. H., Jr. 1995. Apolipoprotein E epsilon 4 allele is not associated with earlier age at onset in amyotrophic lateral sclerosis. *Ann Neurol*, **38**, 460-3.
- Mulder, D. W. & Howard, F. M., Jr. 1976. Patient resistance and prognosis in amyotrophic lateral sclerosis. *Mayo Clin Proc*, **51**, 537-41.
- Neuville, P., Bochaton-Piallat, M. L. & Gabbiani, G. 2000. Retinoids and arterial smooth muscle cells. *Arterioscler Thromb Vasc Biol*, **20**, 1882-8.
- Nguyen, Q. T., Parsadanian, A. S., Snider, W. D. & Lichtman, J. W. 1998. Hyperinnervation of neuromuscular junctions caused by GDNF overexpression in muscle. *Science*, **279**, 1725-9.
- Nie, D. Y., Zhou, Z. H., Ang, B. T., Teng, F. Y., Xu, G., Xiang, T., Wang, C. Y., Zeng, L., Takeda, Y., Xu, T. L., Ng, Y. K., Faivre-Sarrailh, C., Popko, B., Ling, E. A., Schachner, M., Watanabe, K., Pallen, C. J., Tang, B. L. & Xiao, Z. C. 2003. Nogo-A at CNS paranodes is a ligand of Caspr: possible regulation of K(+) channel localization. *Embo J*, **22**, 5666-78.
- Nishimura, A. L., Mitne-Neto, M., Silva, H. C., Richieri-Costa, A., Middleton, S., Cascio, D., Kok, F., Oliveira, J. R., Gillingwater, T., Webb, J., Skehel, P. & Zatz, M. 2004. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am J Hum Genet*, **75**, 822-31.
- Norris, F., Shepherd, R., Denys, E., U, K., Mukai, E., Elias, L., Holden, D. & Norris, H. 1993. Onset, natural history and outcome in idiopathic adult motor neuron disease. *J Neurol Sci*, **118**, 48-55.
- Norris, F. H., Jr., Calanchini, P. R., Fallat, R. J., Panchari, S. & Jewett, B. 1974. The administration of guanidine in amyotrophic lateral sclerosis. *Neurology*, **24**, 721-8.

- Novak, G., Kim, D., Seeman, P. & Tallero, T. 2002. Schizophrenia and Nogo: elevated mRNA in cortex, and high prevalence of a homozygous CAA insert. *Brain Res Mol Brain Res*, **107**, 183-9.
- Oba, H., Araki, T., Ohtomo, K., Monzawa, S., Uchiyama, G., Koizumi, K., Nogata, Y., Kachi, K., Shiozawa, Z. & Kobayashi, M. 1993. Amyotrophic lateral sclerosis: T2 shortening in motor cortex at MR imaging. *Radiology*, **189**, 843-6.
- Oertle, T., Huber, C., van der Putten, H. & Schwab, M. E. 2003a. Genomic structure and functional characterisation of the promoters of human and mouse nogo/rtn4. *J Mol Biol*, **325**, 299-323.
- Oertle, T., Klinger, M., Stuermer, C. A. & Schwab, M. E. 2003b. A reticular rhapsody: phylogenetic evolution and nomenclature of the RTN/Nogo gene family. *Faseb J*, **17**, 1238-47.
- Oertle, T., Merkler, D. & Schwab, M. E. 2003c. Do cancer cells die because of Nogo-B? *Oncogene*, **22**, 1390-9.
- Oertle, T. & Schwab, M. E. 2003. Nogo and its paRTNers. *Trends Cell Biol*, **13**, 187-94.
- Oertle, T., van der Haar, M. E., Bandtlow, C. E., Robeva, A., Burfeind, P., Buss, A., Huber, A. B., Simonen, M., Schnell, L., Brosamle, C., Kaupmann, K., Vallon, R. & Schwab, M. E. 2003d. Nogo-A inhibits neurite outgrowth and cell spreading with three discrete regions. *J Neurosci*, **23**, 5393-406.
- Ogawa, Y., Kosaka, H., Nakanishi, T., Shimizu, A., Ohoi, N., Shouji, H., Yanagihara, T. & Sakoda, S. 1997. Stability of mutant superoxide dismutase-1 associated with familial amyotrophic lateral sclerosis determines the manner of copper release and induction of thioredoxin in erythrocytes. *Biochem Biophys Res Commun*, **241**, 251-7.
- Orrell, R. W., King, A. W., Lane, R. J. & de Belleruche, J. S. 1995. Investigation of a null mutation of the CNTF gene in familial amyotrophic lateral sclerosis. *J Neurol Sci*, **132**, 126-8.
- Parboosingh, J. S., Meininger, V., McKenna-Yasek, D., Brown, R. H., Jr. & Rouleau, G. A. 1999. Deletions causing spinal muscular atrophy do not predispose to amyotrophic lateral sclerosis. *Arch Neurol*, **56**, 710-2.
- Park, J. B., Yiu, G., Kaneko, S., Wang, J., Chang, J., He, X. L., Garcia, K. C. & He, Z. 2005. A TNF receptor family member, TROY, is a coreceptor with Nogo receptor in mediating the inhibitory activity of myelin inhibitors. *Neuron*, **45**, 345-51.
- Perou, C. M., Sorlie, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., Rees, C. A., Pollack, J. R., Ross, D. T., Johnsen, H., Akslen, L. A., Fluge, O., Pergamenschikov, A., Williams, C., Zhu, S. X., Lonning, P. E., Borresen-Dale, A. L., Brown, P. O. & Botstein, D. 2000. Molecular portraits of human breast tumours. *Nature*, **406**, 747-52.

- Pilegaard, H., Ordway, G. A., Saltin, B. & Neufer, P. D. 2000. Transcriptional regulation of gene expression in human skeletal muscle during recovery from exercise. *Am J Physiol Endocrinol Metab*, **279**, E806-14.
- Pinter, M. J., Waldeck, R. F., Wallace, N. & Cork, L. C. 1995. Motor unit behavior in canine motor neuron disease. *J Neurosci*, **15**, 3447-57.
- Pioro, E. P., Antel, J. P., Cashman, N. R. & Arnold, D. L. 1994. Detection of cortical neuron loss in motor neuron disease by proton magnetic resonance spectroscopic imaging in vivo. *Neurology*, **44**, 1933-8.
- Poloni, M., Facchetti, D., Mai, R., Micheli, A., Agnoletti, L., Francolini, G., Mora, G., Camana, C., Mazzini, L. & Bachetti, T. 2000. Circulating levels of tumour necrosis factor-alpha and its soluble receptors are increased in the blood of patients with amyotrophic lateral sclerosis. *Neurosci Lett*, **287**, 211-4.
- Practice advisory on the treatment of amyotrophic lateral sclerosis with riluzole. 1997. Practice advisory on the treatment of amyotrophic lateral sclerosis with riluzole: report of the Quality Standards Subcommittee of the American Academy of Neurology. *Neurology*, **49**, 657-9.
- Pramatarova, A., Laganriere, J., Roussel, J., Brisebois, K. & Rouleau, G. A. 2001. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J Neurosci*, **21**, 3369-74.
- Qi, B., Qi, Y., Watari, A., Yoshioka, N., Inoue, H., Minemoto, Y., Yamashita, K., Sasagawa, T. & Yutsudo, M. 2003. Pro-apoptotic ASY/Nogo-B protein associates with ASYIP. *J Cell Physiol*, **196**, 312-8.
- Rabin, B. A., Griffin, J. W., Crain, B. J., Scavina, M., Chance, P. F. & Cornblath, D. R. 1999. Autosomal dominant juvenile amyotrophic lateral sclerosis. *Brain*, **122 (Pt 8)**, 1539-50.
- Ralph, G. S., Radcliffe, P. A., Day, D. M., Carthy, J. M., Leroux, M. A., Lee, D. C., Wong, L. F., Bilstrand, L. G., Greensmith, L., Kingsman, S. M., Mitrophanous, K. A., Mazarakis, N. D. & Azzouz, M. 2005. Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. *Nat Med*, **11**, 429-33.
- Reed, D. M. & Brody, J. A. 1975. Amyotrophic lateral sclerosis and parkinsonism-dementia on Guam, 1945-1972. I. Descriptive epidemiology. *Am J Epidemiol*, **101**, 287-301.
- Reed, D. M., Torres, J. M. & Brody, J. A. 1975. Amyotrophic lateral sclerosis and parkinsonism-dementia on Guam, 1945-1972. II. Familial and genetic studies. *Am J Epidemiol*, **101**, 302-10.
- Reindl, M., Khantane, S., Ehling, R., Schanda, K., Lutterotti, A., Brinkhoff, C., Oertle, T., Schwab, M. E., Deisenhammer, F., Berger, T. & Bandtlow, C. E. 2003. Serum and cerebrospinal fluid antibodies to Nogo-A in patients with multiple sclerosis and acute neurological disorders. *J Neuroimmunol*, **145**, 139-47.

- Ripps, M. E., Huntley, G. W., Hof, P. R., Morrison, J. H. & Gordon, J. W. 1995. Transgenic mice expressing an altered murine superoxide dismutase gene provide an animal model of amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A*, **92**, 689-93.
- Riviere, M., Meininger, V., Zeisser, P. & Munsat, T. 1998. An analysis of extended survival in patients with amyotrophic lateral sclerosis treated with riluzole. *Arch Neurol*, **55**, 526-8.
- Roebroek, A. J., van de Velde, H. J., Van Bokhoven, A., Broers, J. L., Ramaekers, F. C. & Van de Ven, W. J. 1993. Cloning and expression of alternative transcripts of a novel neuroendocrine-specific gene and identification of its 135-kDa translational product. *J Biol Chem*, **268**, 13439-47.
- Rosen, D. R. 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature*, **364**, 362.
- Sagot, Y., Dubois-Dauphin, M., Tan, S. A., de Bilbao, F., Aebischer, P., Martinou, J. C. & Kato, A. C. 1995. Bcl-2 overexpression prevents motoneuron cell body loss but not axonal degeneration in a mouse model of a neurodegenerative disease. *J Neurosci*, **15**, 7727-33.
- Sagot, Y., Tan, S. A., Hammang, J. P., Aebischer, P. & Kato, A. C. 1996. GDNF slows loss of motoneurons but not axonal degeneration or premature death of pmn/pmn mice. *J Neurosci*, **16**, 2335-41.
- Santos, A. F. & Caroni, P. 2003. Assembly, plasticity and selective vulnerability to disease of mouse neuromuscular junctions. *J Neurocytol*, **32**, 849-62.
- Schena, M. 2002. Microarray analysis. *New York J. Wiley & Sons*.
- Schuler, G. D. 1997. Pieces of the puzzle: expressed sequence tags and the catalog of human genes. *J Mol Med*, **75**, 694-8.
- Schweigreiter, R., Walmsley, A. R., Niederost, B., Zimmermann, D. R., Oertle, T., Casademunt, E., Frentzel, S., Dechant, G., Mir, A. & Bandtlow, C. E. 2004. Versican V2 and the central inhibitory domain of Nogo-A inhibit neurite growth via p75NTR/NgR-independent pathways that converge at RhoA. *Mol Cell Neurosci*, **27**, 163-74.
- Segawa, F. 1993. [MR findings of the pyramidal tract in amyotrophic lateral sclerosis]. *Rinsho Shinkeigaku*, **33**, 835-44.
- Senden, N., Linnoila, I., Timmer, E., van de Velde, H., Roebroek, A., Van de Ven, W., Broers, J. & Ramaekers, F. 1997a. Neuroendocrine-specific protein (NSP)-reticulons as independent markers for non-small cell lung cancer with neuroendocrine differentiation. An in vitro histochemical study. *Histochem Cell Biol*, **108**, 155-65.
- Senden, N. H., Timmer, E. D., Boers, J. E., van de Velde, H. J., Roebroek, A. J., Van de Ven, W. J., Broers, J. L. & Ramaekers, F. C. 1996. Neuroendocrine-specific

- protein C (NSP-C): subcellular localization and differential expression in relation to NSP-A. *Eur J Cell Biol*, **69**, 197-213.
- Senden, N. H., Timmer, E. D., de Bruine, A., Wagenaar, S. S., Van de Velde, H. J., Roebroek, A. J., Van de Ven, W. J., Broers, J. L. & Ramaekers, F. C. 1997b. A comparison of NSP-reticulons with conventional neuroendocrine markers in immunophenotyping of lung cancers. *J Pathol*, **182**, 13-21.
- Senden, N. H., van de Velde, H. J., Broers, J. L., Timmer, E. D., Kuijpers, H. J., Roebroek, A. J., Van de Ven, W. J. & Ramaekers, F. C. 1994a. Subcellular localization and supramolecular organization of neuroendocrine-specific protein B (NSP-B) in small cell lung cancer. *Eur J Cell Biol*, **65**, 341-53.
- Senden, N. H., van de Velde, H. J., Broers, J. L., Timmer, E. D., Roebroek, A. J., van de Ven, W. J. & Ramaekers, F. C. 1994b. Cluster-10 lung-cancer antibodies recognize NSPs, novel neuro-endocrine proteins associated with membranes of the endoplasmic reticulum. *Int J Cancer Suppl*, **8**, 84-8.
- Sengun, I. S. & Appel, S. H. 2003. Serum anti-Fas antibody levels in amyotrophic lateral sclerosis. *J Neuroimmunol*, **142**, 137-40.
- Shao, Z., Browning, J. L., Lee, X., Scott, M. L., Shulga-Morskaya, S., Allaire, N., Thill, G., Levesque, M., Sah, D., McCoy, J. M., Murray, B., Jung, V., Pepinsky, R. B. & Mi, S. 2005. TAJ/TROY, an orphan TNF receptor family member, binds Nogo-66 receptor 1 and regulates axonal regeneration. *Neuron*, **45**, 353-9.
- Shaw, P. J., Forrest, V., Ince, P. G., Richardson, J. P. & Wastell, H. J. 1995. CSF and plasma amino acid levels in motor neuron disease: elevation of CSF glutamate in a subset of patients. *Neurodegeneration*, **4**, 209-16.
- Shefner, J. M., Cudkowicz, M. E., Schoenfeld, D., Conrad, T., Taft, J., Chilton, M., Urbinelli, L., Qureshi, M., Zhang, H., Pestronk, A., Caress, J., Donofrio, P., Sorenson, E., Bradley, W., Lomen-Hoerth, C., Pioro, E., Reznia, K., Ross, M., Pascuzzi, R., Heiman-Patterson, T., Tandan, R., Mitsumoto, H., Rothstein, J., Smith-Palmer, T., MacDonald, D. & Burke, D. 2004. A clinical trial of creatine in ALS. *Neurology*, **63**, 1656-61.
- Shin, Y. J., Woo, J. H., Chung, C. H. & Kim, H. S. 2000. Retinoic acid and its geometrical isomers block both growth and fusion of L6 myoblasts by modulating the expression of protein kinase A. *Mol Cells*, **10**, 162-8.
- Shipp, M. A., Ross, K. N., Tamayo, P., Weng, A. P., Kutok, J. L., Aguiar, R. C., Gaasenbeek, M., Angelo, M., Reich, M., Pinkus, G. S., Ray, T. S., Koval, M. A., Last, K. W., Norton, A., Lister, T. A., Mesirov, J., Neuberg, D. S., Lander, E. S., Aster, J. C. & Golub, T. R. 2002. Diffuse large B-cell lymphoma outcome prediction by gene-expression profiling and supervised machine learning. *Nat Med*, **8**, 68-74.
- Siciliano, G., Pastorini, E., Pasquali, L., Manca, M. L., Iudice, A. & Murri, L. 2001. Impaired oxidative metabolism in exercising muscle from ALS patients. *J Neurol Sci*, **191**, 61-5.

- Simonen, M., Pedersen, V., Weinmann, O., Schnell, L., Buss, A., Ledermann, B., Christ, F., Sansig, G., van der Putten, H. & Schwab, M. E. 2003. Systemic deletion of the myelin-associated outgrowth inhibitor Nogo-A improves regenerative and plastic responses after spinal cord injury. *Neuron*, **38**, 201-11.
- Simpson, E. P., Henry, Y. K., Henkel, J. S., Smith, R. G. & Appel, S. H. 2004. Increased lipid peroxidation in sera of ALS patients: a potential biomarker of disease burden. *Neurology*, **62**, 1758-65.
- Simpson, E. P., Yen, A. A. & Appel, S. H. 2003. Oxidative Stress: a common denominator in the pathogenesis of amyotrophic lateral sclerosis. *Curr Opin Rheumatol*, **15**, 730-6.
- Sironen, R. K., Karjalainen, H. M., Torronen, K. J., Elo, M. A., Hyttinen, M. M., Helminen, H. J. & Lammi, M. J. 2004. Reticulon 4 in chondrocytic cells: barosensitivity and intracellular localization. *Int J Biochem Cell Biol*, **36**, 1521-31.
- Smith, R. G., Haverkamp, L. J., Case, S., Appel, V. & Appel, S. H. 1996. Apolipoprotein E epsilon 4 in bulbar-onset motor neuron disease. *Lancet*, **348**, 334-5.
- Smith, R. G., Henry, Y. K., Mattson, M. P. & Appel, S. H. 1998. Presence of 4-hydroxynonenal in cerebrospinal fluid of patients with sporadic amyotrophic lateral sclerosis. *Ann Neurol*, **44**, 696-9.
- Sorlie, T., Perou, C. M., Tibshirani, R., Aas, T., Geisler, S., Johnsen, H., Hastie, T., Eisen, M. B., van de Rijn, M., Jeffrey, S. S., Thorsen, T., Quist, H., Matese, J. C., Brown, P. O., Botstein, D., Eystein Lonning, P. & Borresen-Dale, A. L. 2001. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. *Proc Natl Acad Sci U S A*, **98**, 10869-74.
- Spencer, P. S., Nunn, P. B., Hugon, J., Ludolph, A. C., Ross, S. M., Roy, D. N. & Robertson, R. C. 1987. Guam amyotrophic lateral sclerosis-parkinsonism-dementia linked to a plant excitant neurotoxin. *Science*, **237**, 517-22.
- Spreux-Varoquaux, O., Bensimon, G., Lacomblez, L., Salachas, F., Pradat, P. F., Le Forestier, N., Marouan, A., Dib, M. & Meininger, V. 2002. Glutamate levels in cerebrospinal fluid in amyotrophic lateral sclerosis: a reappraisal using a new HPLC method with coulometric detection in a large cohort of patients. *J Neurol Sci*, **193**, 73-8.
- Steiner, P., Kulangara, K., Sarria, J. C., Glauser, L., Regazzi, R. & Hirling, H. 2004. Reticulon 1-C/neuroendocrine-specific protein-C interacts with SNARE proteins. *J Neurochem*, **89**, 569-80.
- Stevenson, E. J., Giresi, P. G., Koncarevic, A. & Kandarian, S. C. 2003. Global analysis of gene expression patterns during disuse atrophy in rat skeletal muscle. *J Physiol*, **551**, 33-48.
- Storkebaum, E., Lambrechts, D., Dewerchin, M., Moreno-Murciano, M. P., Appelmans, S., Oh, H., Van Damme, P., Rutten, B., Man, W. Y., De Mol, M.,

- Wyns, S., Manka, D., Vermeulen, K., Van Den Bosch, L., Mertens, N., Schmitz, C., Robberecht, W., Conway, E. M., Collen, D., Moons, L. & Carmeliet, P. 2005. Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. *Nat Neurosci*, **8**, 85-92.
- Swash, M. 1998. Early diagnosis of ALS/MND. *J Neurol Sci*, **160 Suppl 1**, S33-6.
- Tagami, S., Eguchi, Y., Kinoshita, M., Takeda, M. & Tsujimoto, Y. 2000. A novel protein, RTN-XS, interacts with both Bcl-XL and Bcl-2 on endoplasmic reticulum and reduces their anti-apoptotic activity. *Oncogene*, **19**, 5736-46.
- Takahashi, R. 1995. [Deficiency of human ciliary neurotropic factor (CNTF) is not causally related to amyotrophic lateral sclerosis (ALS)]. *Rinsho Shinkeigaku*, **35**, 1543-5.
- Takahashi, R., Yokoji, H., Misawa, H., Hayashi, M., Hu, J. & Deguchi, T. 1994. A null mutation in the human CNTF gene is not causally related to neurological diseases. *Nat Genet*, **7**, 79-84.
- Taketomi, M., Kinoshita, N., Kimura, K., Kitada, M., Noda, T., Asou, H., Nakamura, T. & Ide, C. 2002. Nogo-A expression in mature oligodendrocytes of rat spinal cord in association with specific molecules. *Neurosci Lett*, **332**, 37-40.
- Tambe, Y., Isono, T., Haraguchi, S., Yoshioka-Yamashita, A., Yutsudo, M. & Inoue, H. 2004. A novel apoptotic pathway induced by the drs tumor suppressor gene. *Oncogene*, **23**, 2977-87.
- Tanabe, K., Hirade, K., Ishisaki, A., Shu, E., Suga, H., Kitajima, Y., Katagiri, Y., Dohi, S. & Kozawa, O. 2004. Possible involvement of p44/p42 MAP kinase in retinoic acid-stimulated vascular endothelial growth factor release in aortic smooth muscle cells. *Atherosclerosis*, **175**, 245-51.
- The ALS CNTF treatment study (ACTS) phase I-II Study Group. 1996. The Amyotrophic Lateral Sclerosis Functional Rating Scale. Assessment of activities of daily living in patients with amyotrophic lateral sclerosis. *Arch Neurol*, **53**, 141-7.
- The National Isometric Muscle Strength (NIMS) Database Consortium. 1996. Muscular weakness assessment: use of normal isometric strength data. *Arch Phys Med Rehabil*, **77**, 1251-1255.
- Trotti, D., Rolfs, A., Danbolt, N. C., Brown, R. H., Jr. & Hediger, M. A. 1999. SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. *Nat Neurosci*, **2**, 848.
- Udaka, F., Sawada, H., Seriu, N., Shindou, K., Nishitani, N. & Kameyama, M. 1992. MRI and SPECT findings in amyotrophic lateral sclerosis. Demonstration of upper motor neurone involvement by clinical neuroimaging. *Neuroradiology*, **34**, 389-93.

- Ueyama, H., Kumamoto, T., Fujimoto, S., Murakami, T. & Tsuda, T. 1998. Expression of three calpain isoform genes in human skeletal muscles. *J Neurol Sci*, **155**, 163-9.
- van de Velde, H. J., Roebroek, A. J., Senden, N. H., Ramaekers, F. C. & Van de Ven, W. J. 1994a. NSP-encoded reticulons, neuroendocrine proteins of a novel gene family associated with membranes of the endoplasmic reticulum. *J Cell Sci*, **107 (Pt 9)**, 2403-16.
- van de Velde, H. J., Senden, N. H., Roskams, T. A., Broers, J. L., Ramaekers, F. C., Roebroek, A. J. & Van de Ven, W. J. 1994b. NSP-encoded reticulons are neuroendocrine markers of a novel category in human lung cancer diagnosis. *Cancer Res*, **54**, 4769-76.
- van 't Veer, L. J., Dai, H., van de Vijver, M. J., He, Y. D., Hart, A. A., Mao, M., Peterse, H. L., van der Kooy, K., Marton, M. J., Witteveen, A. T., Schreiber, G. J., Kerkhoven, R. M., Roberts, C., Linsley, P. S., Bernards, R. & Friend, S. H. 2002. Gene expression profiling predicts clinical outcome of breast cancer. *Nature*, **415**, 530-6.
- Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J. S. & Lowell, B. B. 1997. UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem Biophys Res Commun*, **235**, 79-82.
- Vidal-Puig, A. J., Grujic, D., Zhang, C. Y., Hagen, T., Boss, O., Ido, Y., Szczepanik, A., Wade, J., Mootha, V., Cortright, R., Muoio, D. M. & Lowell, B. B. 2000. Energy metabolism in uncoupling protein 3 gene knockout mice. *J Biol Chem*, **275**, 16258-66.
- Vielhaber, S., Kunz, D., Winkler, K., Wiedemann, F. R., Kirches, E., Feistner, H., Heinze, H. J., Elger, C. E., Schubert, W. & Kunz, W. S. 2000. Mitochondrial DNA abnormalities in skeletal muscle of patients with sporadic amyotrophic lateral sclerosis. *Brain*, **123 (Pt 7)**, 1339-48.
- Vielhaber, S., Winkler, K., Kirches, E., Kunz, D., Buchner, M., Feistner, H., Elger, C. E., Ludolph, A. C., Riepe, M. W. & Kunz, W. S. 1999. Visualization of defective mitochondrial function in skeletal muscle fibers of patients with sporadic amyotrophic lateral sclerosis. *J Neurol Sci*, **169**, 133-9.
- Visser, J., Mans, E., de Visser, M., van den Berg-Vos, R. M., Franssen, H., de Jong, J. M., van den Berg, L. H., Wokke, J. H. & de Haan, R. J. 2003. Comparison of maximal voluntary isometric contraction and hand-held dynamometry in measuring muscle strength of patients with progressive lower motor neuron syndrome. *Neuromuscul Disord*, **13**, 744-50.
- Wakana, Y., Koyama, S., Nakajima, K., Hatsuzawa, K., Nagahama, M., Tani, K., Hauri, H. P., Melancon, P. & Tagaya, M. 2005. Reticulon 3 is involved in membrane trafficking between the endoplasmic reticulum and Golgi. *Biochem Biophys Res Commun*, **334**, 1198-205.

- Wang, K. C., Kim, J. A., Sivasankaran, R., Segal, R. & He, Z. 2002a. P75 interacts with the Nogo receptor as a co-receptor for Nogo, MAG and OMgp. *Nature*, **420**, 74-8.
- Wang, L. J., Lu, Y. Y., Muramatsu, S., Ikeguchi, K., Fujimoto, K., Okada, T., Mizukami, H., Matsushita, T., Hanazono, Y., Kume, A., Nagatsu, T., Ozawa, K. & Nakano, I. 2002b. Neuroprotective effects of glial cell line-derived neurotrophic factor mediated by an adeno-associated virus vector in a transgenic animal model of amyotrophic lateral sclerosis. *J Neurosci*, **22**, 6920-8.
- Wieczorek, D. F. & Hughes, S. R. 1991. Developmentally regulated cDNA expressed exclusively in neural tissue. *Brain Res Mol Brain Res*, **10**, 33-41.
- Wiedemann, F. R., Winkler, K., Kuznetsov, A. V., Bartels, C., Vielhaber, S., Feistner, H. & Kunz, W. S. 1998. Impairment of mitochondrial function in skeletal muscle of patients with amyotrophic lateral sclerosis. *J Neurol Sci*, **156**, 65-72.
- Wilbourn, A. J. 1998. Clinical neurophysiology in the diagnosis of amyotrophic lateral sclerosis: the Lambert and the El Escorial criteria. *J Neurol Sci*, **160 Suppl 1**, S25-9.
- Williamson, T. L., Bruijn, L. I., Zhu, Q., Anderson, K. L., Anderson, S. D., Julien, J. P. & Cleveland, D. W. 1998. Absence of neurofilaments reduces the selective vulnerability of motor neurons and slows disease caused by a familial amyotrophic lateral sclerosis-linked superoxide dismutase 1 mutant. *Proc Natl Acad Sci U S A*, **95**, 9631-6.
- Williamson, T. L. & Cleveland, D. W. 1999. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat Neurosci*, **2**, 50-6.
- Wixon, J. & Kell, D. 2000. The Kyoto encyclopedia of genes and genomes--KEGG. *Yeast*, **17**, 48-55.
- Wong, P. C., Pardo, C. A., Borchelt, D. R., Lee, M. K., Copeland, N. G., Jenkins, N. A., Sisodia, S. S., Cleveland, D. W. & Price, D. L. 1995. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron*, **14**, 1105-16.
- Xiao, Y., Grieshammer, U. & Rosenthal, N. 1995. Regulation of a muscle-specific transgene by retinoic acid. *J Cell Biol*, **129**, 1345-54.
- Yagishita, A. 1995. [MR imaging of the brain of amyotrophic lateral sclerosis]. *Rinsho Shinkeigaku*, **35**, 1554-6.
- Yamamoto, A., Lucas, J. J. & Hen, R. 2000. Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. *Cell*, **101**, 57-66.
- Yang, Y., Hentati, A., Deng, H. X., Dabbagh, O., Sasaki, T., Hirano, M., Hung, W. Y., Ouahchi, K., Yan, J., Azim, A. C., Cole, N., Gascon, G., Yagmour, A., Ben-Hamida, M., Pericak-Vance, M., Hentati, F. & Siddique, T. 2001. The gene

encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat Genet*, **29**, 160-5.

Yoo, H. Y., Chang, M. S. & Rho, H. M. 1999. Induction of the rat Cu/Zn superoxide dismutase gene through the peroxisome proliferator-responsive element by arachidonic acid. *Gene*, **234**, 87-91.

Yoshihara, T., Ishii, T., Iwata, M. & Nomoto, M. 1998. Ultrastructural and histochemical study of the motor end plates of the intrinsic laryngeal muscles in amyotrophic lateral sclerosis. *Ultrastruct Pathol*, **22**, 121-6.

Zheng, B., Ho, C., Li, S., Keirstead, H., Steward, O. & Tessier-Lavigne, M. 2003. Lack of enhanced spinal regeneration in Nogo-deficient mice. *Neuron*, **38**, 213-24.

Zhu, S., Stavrovskaya, I. G., Drozda, M., Kim, B. Y., Ona, V., Li, M., Sarang, S., Liu, A. S., Hartley, D. M., Wu du, C., Gullans, S., Ferrante, R. J., Przedborski, S., Kristal, B. S. & Friedlander, R. M. 2002. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature*, **417**, 74-8.

a. Kandel, E.R., Schwartz J.H. & Jessel T.M. Principles of neural science. *McGraw-Hill, Fourth Edition*, Figure 33-12 page 667 (2000).

Curriculum vitae

Franck Di Scala

12, rue Joseph Guerber
67100 Strasbourg
France

Born on December 12th 1977 in Mulhouse, France

Phone : +33 (0)6 63 21 10 77

E-mail : franckdiscala@free.fr

Research Experience

2001-2005 **PhD in biology**

Topic : Analysis of skeletal muscle in amyotrophic lateral sclerosis: etiological, diagnostic and therapeutic aspects

Techniques : RT-PCR, Northern blot, Western blot, ELISA, cellular culture, screening with DNA microarray and bioinformatic analysis

Supervision : Dr. Jean-Philippe Loeffler and Prof. Mickael Primig, Inserm U692 SMN in Strasbourg and Biozentrum in Basel, collaboration with Pitié-Salpêtrière hospital in Paris

Results : 5 international publications, 2 patents, 2 articles in specialized papers

2001-2001 **Training course of the DEA/Postgraduate's degree** "Study of a gene inhibiting axonal regeneration in a transgenic model of Amyotrophic Lateral Sclerosis", under the supervision of Dr. Jean-Philippe Loeffler, Inserm U692 SMN in Strasbourg

1999-2000 2-months training course of Master's degree and 2-months training course of Bachelor's degree

Teaching Experience

2001-2005 Teaching of cellular biology for students in first year of biology

2004 Organizer of the Inserm training course "DNA microarray", IGBMC/Genopole in Illkirch

2003 Teaching for Louis Pasteur University training course "Molecular bases and technical approaches in the study of apoptosis", Strasbourg

2002 Teaching for Master's degree trainees, Strasbourg

Education

2001 Postgraduate's Degree, Louis Pasteur University of Strasbourg

2000 Master's degree in Biology, Louis Pasteur University of Strasbourg

1999 Bachelor's degree in Biology, Louis Pasteur University of Strasbourg

1998 Second year in Biology, Louis Pasteur University of Strasbourg

1996 First year of Medical studies, Louis Pasteur University of Strasbourg

1992 Baccalauréat/High-School degree in Biology, Mulhouse

Publications

Di Scala F, Dupuis L, Gaiddon C, de Tapia M, Jokic N, Gonzalez De Aguilar JL, Raul JS, Ludes B, Loeffler JP. *Tissue specificity and regulation of the amino-terminal diversity of reticulon 3*. Biochem J. 2005 Jan 1;385(Pt 1):125-34.

Jokic N, Di Scala F, Dupuis L, Rene F, Muller A, De Aguilar JL, Loeffler JP. *Early activation of antioxidant mechanisms in muscle of mutant Cu/Zn-superoxide dismutase-linked amyotrophic lateral sclerosis mice*. Ann N Y Acad Sci. 2003 Dec;1010:552-6.

Dupuis L, di Scala F, Rene F, de Tapia M, Oudart H, Pradat PF, Meininger V, Loeffler JP. *Up-regulation of mitochondrial uncoupling protein 3 reveals an early muscular metabolic defect in amyotrophic lateral sclerosis*. FASEB J. 2003 Nov;17(14):2091-3.

Dupuis L, Rene F, Di Scala F, Gonzalez De Aguilar JL, De Tapia M, Loeffler JP. *Denervation is not a primary cause of prion protein down-regulation occurring in the spinal cord of a transgenic model of amyotrophic lateral sclerosis*. Ann N Y Acad Sci. 2002 Nov;973:116-9.

Dupuis L, Gonzalez de Aguilar JL, di Scala F, Rene F, de Tapia M, Pradat PF, Lacomblez L, Seihlan D, Prinjha R, Walsh FS, Meininger V, Loeffler JP. *Nogo provides a molecular marker for diagnosis of amyotrophic lateral sclerosis*. Neurobiol Dis. 2002 Aug;10(3):358-65.

Conferences participation with posters

International Society for Neurochemistry Conference, Avignon, May 13th - 16th 2004.

Journées de Neurologie de Langue Française, Strasbourg, 13 - 17 avril 2004.

Apoptosis 2003, Luxembourg, January 29th - February 1st 2003.

Federation of European Neuroscience Societies Forum, Paris, July 13th - 17th 2002.

Neurex 2001 meeting, Strasbourg, December 11th 2001.

Neurex 2000 meeting, Freiburg, December 8th 2000.

Patents

Dupuis L, Di Scala F, De Tapia M, Larmet Y, Loeffler JP. *Use of mitochondrial uncoupling proteins for diagnostic, prevention and treatment of diseases involving neuromuscular affection*. Patent n°03291522.5. Filing Date 2003-06-20.

Gaiddon C, Gonzalez De Aguilar JL, De Tapia M, Larmet Y, Di Scala F, Dupuis L, René F, Boutillier AL, Loeffler JP. *Compositions and methods for detecting pathologies affecting neuromuscular transmission*. Patent n° WO2004001069. Publication date 2003-12-31.

Articles in specialized papers

Collaborations in Neurex, sharing resources and technologies for the fight against Amyotrophic Lateral Sclerosis. Eltem-Neurex Newsletter, march 2003, page 12-13.

Les neurosciences : du laboratoire à la clinique. ULP sciences, magazine from Louis Pasteur University of Strasbourg, january 2003 n°10, page 17.

Languages

French : mother tongue

English : fluent, TOEIC score 910, thesis written and defended in english

German : good notions

Computer

Word, Excel, PowerPoint, Canevas, Photoshop, NCBI and DNA microarray databases

Hobbies

Scientific popularization

Tennis and squash

Reading