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(54) Title: METHOD OF TREATMENT OF AMYOTROPHIC LATERAL SCLEROSIS

(57) Abstract: The present invention concerns an agonist of a MCH receptor for use for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS.

Method of treatment of amyotrophic lateral sclerosis

The present invention concerns the treatment of amyotrophic lateral sclerosis, in particular the prevention of weight loss in subjects suffering from amyotrophic lateral sclerosis.

Amyotrophic lateral sclerosis (ALS), sometimes called Lou Gehrig's disease or Charcot disease, is a rapidly progressive, invariably fatal neurological disease affecting motor neurons and nerve cells responsible for controlling voluntary muscles. The disease belongs to a group of disorders known as *motor neuron diseases (MNDs),* which are characterized by the gradual degeneration and death of motor neurons. ALS incidence and prevalence are worldwide respectively 2 and 8/100000 and mostly concerns people over 40. The average life expectancy after the diagnosis is around 2 years, making it one of the most fatal neurological diseases associated with the highest unmet medical need.

Despite efforts in understanding the pathobiology of the disease and identifying new targets, there is no effective treatment today. The only approved drug for ALS is Rilutek (Riluzole), a neuroprotective agent commercialized in 1996. It increases the lifespan from 3 to 6 months only, illustrating the poverty and ineffectiveness of current therapeutic solutions. Apart from Rilutek, the arsenal is completed with more than 30 supplementary off-label drugs, prescribed in order to relieve the myriad of symptoms associated with ALS.

These last years, despite a cohort of various and numerous candidates reaching Phase 2 and 3 clinical trials, all failed to achieve their endpoints, demonstrating the difficulty to address ALS, mainly explained by the fact that ALS is a poorly understood disease.

Motor symptoms of ALS are frequently accompanied by weight loss in most patients. ALS-related weight loss is an early phenomenon, occurring before the onset of motor symptoms in patients. It has been established repeatedly that patients who lose the most weight are those who have shorter life. It was shown, for example, that patients with a body mass index indicative of denutrition exhibited very short survival (Desport, 1999). More recently the same group has shown that a modest weight loss of 5% of initial body weight (or 3-5 kgs) at diagnosis was strongly correlated with very poor prognosis (Marin, 2011). Similar studies have been undertaken in many countries and are all converging. Based on those correlations, nutritional intervention to increase caloric intake has been tested in mouse models, and more recently in a specific population of ALS patients. Indeed, high fat feeding potently delayed neurodegeneration in the standard SOD1 model

of ALS (Dupuis, 2004), while hypercaloric nutrition of ALS patients under gastrostomy yielded encouraging results in a pilot clinical trial (Wills, 2014). This link between weight and disease progression has been the subject of many publication and studies. However, until now, weight loss in ALS patients was not explained in terms of biological mechanisms and consequently, there is no pharmacological approach in development to treat ALS associated weight loss.

The present inventors discovered a mechanism causing unbalanced metabolism and weight loss in ALS. They observed that typical ALS pathology occurs in the lateral hypothalamus in both SOD1 mice and human ALS patients. By comparing SOD1 mice and ALS patients, the inventors identified that MCH (Melanin Concentrating Hormone), a hypothalamic neuropeptide known for its role in appetite stimulation, and weight control, was a potential target for treating weight loss in these patients. They showed that continuous intracerebroventricular (ICV) administration of the peptide leads to weight gain in SOD1 model and no effect on control mice.

Importantly, the inventors further demonstrated that 2 weeks of low dose MCH ICV administration was able to restore normal body weight of mutant SOD1 mice independently of increased food intake.

These results demonstrate that decreased levels of one single neuropeptide expressed in the lateral hypothalamus accounts for weight loss in ALS, thus providing a pharmacological strategy to treat weight loss in ALS patients.

The present invention thus concerns an agonist of a Melanin Concentrating Hormone (MCH) receptor for use for reestablishing a metabolic balance in a subject suffering from amyotrophic lateral sclerosis (ALS).

The present invention also concerns an agonist of a MCH receptor for use in the treatment of ALS for reestablishing a metabolic balance in a subject suffering from ALS.

The present invention also concerns an agonist of a MCH receptor for use for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS.

The present invention further concerns an agonist of a MCH receptor for use in the treatment of ALS for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS.

Another object of the invention concerns an agonist of a MCH receptor for use for prolonging life expectancy of a subject suffering from ALS.

Still another object of the invention concerns an agonist of a MCH receptor for use in the treatment of ALS for prolonging life expectancy of a subject suffering from ALS.

The present invention further concerns an agonist of a MCH receptor for use in the treatment of ALS.

Detailed description of the invention

Agonist of MCH receptor

As used herein, the term "MCH receptor" refers to an integral plasma membrane protein which binds melanin-concentrating hormone and agonists thereof. It encompasses MCHR1 and MCHR2.

MCHR1 is typically 353 amino acids long and it has the highest degree of homology to the somatostatin receptor family. The receptor is highly conserved between human and rodents. Database scanning revealed a second human high-affinity receptor for MCH (MCHR2). Human MCHR1 preferably consists of the sequence SEQ ID NO: 3

MSVGAMKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGGRRWRLPQPAWVEGSSARLWEQATGTG
WMDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSISYINIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCN
NVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISS
TKFRKPSVATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLAFALPFVVITAA
YVRILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTFVYLYNAAISLGYAN
SCLNPFVYIVLCETFRKRLVLSVKPAAQGGQLRAVSNAQTADEERTESKGT.

MCHR2 is typically 340 amino acids long and has low (38%) homology to the MCHR1 receptor. Human MCHR2 preferably consists of the sequence SEQ ID NO: 4

MNPFHASCWNTSAELLNKSWNKEFAYQTASVVDTVILPSMIGIICSTGLVGNILIVFTIIRSRKKTVPDIYICNLAV
ADLVHIVGMPFLIHQWARGGEWVFGGPLCTIITSLDTCNQFACSAIMTVMSVDRYFALVQPFRLTRWRTRYKTI
RINLGLWAASFILALPVWVYSKVIKFKDGVESCAFDLTSPDDVLWYTLYLTITTFFFPLPLILVCYILILCYTWEMY
QQNKDARCCNPSVPKQRVMKLTKMVLVLVVVFILSAAPYHVIQLVNLQMEQPTLAFYVGYYLSICLSYASSSIN
PFLYILLSGNFQKRLPQIQRRATEKEINNMGNTLKSHF.

In higher species, the expression pattern of MCHR2 mRNA is similar to MCHR1 with the highest expression observed in the brain, notably in the frontal cortex, amygdala and nucleus accumbens. Both MCH receptors are also expressed in areas associated with energy homeostasis such as the arcuate nucleus and the ventral medial hypothalamus.

In the context of the invention, the MCH receptor refers to MCHR1 or MCHR2. Preferably, the MCH receptor is MCHR1.

As used herein, the term "agonist of MCH receptor" refers to any compound that can directly or indirectly activate the signal transduction cascade related to an MCH receptor, in particular of MCHR1 or MCHR2, preferably of MCHR1.

Tests and assays for determining whether a compound is an agonist of MCH receptor are well known by the skilled person in the art such as described in An et al. (2001).

Typically, binding of a candidate compound to MCHR1 and MCHR2 can be measured by filtration binding assay. Membranes (10 mg protein) from transiently transfected HEK293-MCHR1 and HEK293-MCHR2 cells can be mixed with 0–9.8 nM ^{125}I-labeled candidate compound in a binding buffer (50 mM Hepes 10 mM $MgCl_2$ 2 mM EGTA; protease inhibitors, 0.1% BSA, pH 7.6). After incubation preferably for 1 h at room temperature, membrane-bound ^{125}I-labeled candidate compound can be separated from the free ^{125}I-labeled candidate compound by filtration through a filter plate on a cell harvester and washed with ice-cold binding buffer supplemented with 80 mM NaCl. Eighty microliters of scintillation liquid can typically be added, and the radioactivity can be counted on a counter.

The agonist of MCH receptor used in the context of the invention may be a MCH peptide or a derivative thereof which retains the ability to activate a MCH receptor, small organic molecules, a nucleic acid encoding a MCH peptide or a derivative thereof which retains the ability to activate a MCH receptor and a MCH peptide expression activator.

Preferably, the agonist of a MCH receptor is selected from the group consisting of a MCH peptide or a derivative thereof which retains the ability to activate a MCH receptor, small organic molecules, a nucleic acid encoding a MCH peptide or a derivative thereof which retains the ability to activate a MCH receptor and a MCH peptide expression activator.

More preferably, the agonist of a MCH receptor is selected from the group consisting of a MCH peptide or a derivative thereof which retains the ability to activate a MCH receptor, a nucleic acid encoding a MCH peptide or a derivative thereof which retains the ability to activate a MCH receptor and a MCH peptide expression activator,

Still more preferably, the agonist of a MCH receptor is a MCH peptide or a derivative thereof which retains the ability to activate a MCH receptor. Most preferably, the agonist of a MCH receptor is a MCH peptide.

Preferably, the agonist of a MCH receptor can pass through the Blood-Brain Barrier.

In the context of the invention, the terms "polypeptide" and "peptide" are used indifferently and refer to native peptides (either proteolysis products or synthetically synthesized peptides) and further to peptidomimetics, such as peptoids and semipeptoids which are peptide analogs, which may have, for example, modifications rendering the peptides more stable while in a body, or more immunogenic. Such modifications include,

but are not limited to, cyclization, N-terminus modification, C-terminus modification, peptide bond modification, including, but not limited to, CH2-NH, CH2-S, CH2-S=O, O=C-NH, CH2-O, CH2-CH2, S=C-NH, CH=CH or CF=CH, backbone modification and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified in Quantitative Drug Design, CA. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992).

According to the invention, a peptide consists of less than 50 amino acids, preferably less than 40 amino acids, more preferably less than 30 amino acids, still preferably less 20 amino acids. More preferably, peptides according to the invention have a length of from about 5 to about 19 amino acids, from about 5 to about 18 amino acids, from about 5 to about 17 amino acids, from about 5 to about 16 amino acids, from about 5 to about 15 amino acids, from about 5 to about 14 amino acids, from about 5 to about 13 amino acids.

As used herein, the term "amino acid" is understood to include: the 20 naturally occurring amino acids *i.e.* alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, praline, serine, threonine, tryptophan, tyrosine, and valine; amino acids harbouring the post-translational modifications which can be found *in vivo* such as hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids.

As intended herein, the peptides of the invention can be substituted by one or more atoms or groups.

By "MCH peptide" is meant herein Melanin Concentrating Hormone, a cyclic 19-amino acid orexigenic hypothalamic peptide of sequence DFDMLRCMLGRVYRPCWQV identified as SEQ ID NO: 1 that is a natural agonist of MCH receptors (MCHR1 and MCHR2) known for its role in appetite stimulation and weight control, Human and rodent MCH are identical. MCH peptide is processed from proMCH. proMCH consists typically of 165 amino acids. Preferably, proMCH consists of the sequence SEQ ID NO: 5

MAKMNLSSYILILTFSLFSQGILLSASKSIRNLDDDMVFNTFRLGKGFQKEDTAEKSVIAPSLEQYKNDESSF
MNEEENKVSKNTGSKHNFLNHGLPLNLAIKGYQALKGSVDFPAENGVQNTESTQEKREIGDEENSAKFPIGRR
DFDMLRCMLGRVYRPCWQV.

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By "derivative of a MCH peptide" is meant herein a molecule comprising the amino acid sequence of a MCH peptide subject to various changes, including, but not limited to, chemical modifications, substitutions, insertions, extensions and deletions where such

changes do not destroy the ability of the peptide to activate a MCH receptor. Derivatives of a MCH peptide thus include a protein comprising a MCH peptide, a fragment of a MCH peptide and a mutant of a MCH peptide, which retain the ability to activate a MCH receptor.

A protein comprising a MCH peptide may be proMCH.

A mutant of a MCH peptide is preferably a peptide having at least 80% identity with the peptide of sequence SEQ ID NO: 1.

Preferably, the percentage of identity relates to the number of identical amino-acids obtained for an optimal paired alignment (*i.e.* the alignment maximizing the number of identical amino-acids) of the sequence of a protein homologous to SEQ ID NO: 1, divided by the total number of amino-acids in SEQ ID NO: 1. Alignment can be performed manually or using computer programs such as the EMBOSS-Needle program (Needleman and Wunsch (1970) J. Mol. Biol. 48:443 - 453). The identity percentage may be computed by producing an overall pair wise alignment based on the Needleman-Wunsch alignment algorithm for finding the optimum alignment (including “holes” or “gaps”) between two sequences over the whole of their length, for example by using Needle, and by using the BLOSUM62 matrix with a penalty for inserting « gaps » of 10 and a penalty of extension of « gaps » of 0.5. Preferably, the percentage of identity according to the invention is at least 85%, more preferably from at least 90%, and even more preferably from at least 95%.

It was demonstrated that the smallest fragment of a MCH peptide which retained its ability to activate a MCH receptor may have 11 contiguous amino acids. Consequently, according to the invention a 'fragment of a MCH peptide' may be 11, 12, 13, 14, 15, 16, 17, 18 contiguous amino acids.

By "retaining the ability of a MCH peptide to activate a MCH receptor" is meant herein that the compound can be bound to MCHR1 or MCHR2 and activate the signaling pathway of these receptors, as defined above.

By "nucleic acid encoding a MCH peptide or a derivative thereof" is meant herein an isolated nucleic acid comprising or consisting of a sequence encoding a MCH peptide, preferably, of SEQ ID NO:1 or encoding a derivative thereof. **SEQ ID NO: 2** shows the nucleotide sequence encoding the full-length MCH peptide.

A “nucleic acid” herein refers to the phosphate ester polymeric form of ribonucleosides (also called "RNA molecule"), deoxyribonucleosides (also called "DNA molecule") or any phosphoester analogs thereof, such as phosphorothioates and thioesters, in either single stranded form or a double-stranded form. In one embodiment,

the polynucleotide is selected from the group constituted of DNA, RNA, genomic DNA and cDNA.

Nucleic acids may be produced recombinantly, synthetically, or by any means available to those of skill in the art. They may also be cloned by standard techniques such as PCR (polymerase chain reaction).

In one embodiment, the nucleic acid of the invention contains only a coding region for the MCH peptide of the invention or a derivative thereof. However, in some embodiments, the nucleic acid further comprises, in operable linkage, a portion of nucleic acid that allows for efficient translation of the coding sequence in the target cell. Accordingly, in one embodiment, the nucleic acid (when in a DNA form) further comprises a promoter in operable linkage which allows for the transcription of the coding region and the portion of nucleic acid that allows for efficient translation of the coding region in the target cell. Those elements are further defined in the section « vector » herein below.

Variant nucleic acid sequences may include nucleic acid sequences that have at least about 80% nucleic acid sequence identity with a nucleic acid sequence disclosed herein. Preferably, a variant nucleic acid sequences will have at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% nucleic acid sequence identity to a nucleic acid sequence or a fragment of a nucleic acid sequence as disclosed herein. Nucleic acid sequence identity is defined as the percentage of nucleic acids in the variant sequence that are identical with the nucleic acids in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Sequence identity may be determined over the full length of both variant and reference nucleic acid sequences.

In context of the present invention “vector” refers to vectors used in recombinant DNA techniques allowing entities, such as a nucleic acids, to be transferred into a target cell for the purpose of replicating the vectors comprising the nucleotide sequences used in the invention and/or expressing the proteins encoded by the nucleic acids of the invention. Examples of vectors used in recombinant DNA techniques include but are not limited to plasmids, chromosomes, artificial chromosomes or viruses.

Accordingly, in one embodiment the vector is also a so-called “delivery system”. The vector is preferably an isolated vector.

In a further embodiment the vector is an expression vector.

An “expression vector” herein refers to a vector as defined above designed for protein expression in cells. The vector is engineered to contain regulatory sequences that

act as enhancer and promoter regions and lead to efficient transcription of the gene carried on the expression vector. Those regulatory sequences may as well be called “expression signal” or “control sequences”. Accordingly, in one embodiment, a nucleic acid in a vector is operably linked to a control sequence that is capable of providing for the expression of the coding sequence by the target cell.

The term "operably linked" means that the components described are in a relationship permitting them to function in their intended manner. A regulatory sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences.

In one embodiment, the vector preferably comprises a MCHR agonist expression cassette, *i.e.* a nucleic acid encoding a MCHR agonist of the invention operably linked to at least one expression signal allowing its expression.

The at least one expression signal is particularly selected among a promoter, a terminator, an enhancer and their combinations, preferably among a promoter, a terminator. Suitable promoters, terminators and enhancers are well-known by the skilled person and may be selected to be compatible with the target cell for which the expression vector is designed to be used in.

The control sequences may be modified, for example by the addition of further transcriptional regulatory elements to make the level of transcription directed by the control sequences more responsive to transcriptional modulators.

The term "promoter" is well-known in the art and encompasses nucleic acid regions ranging in size and complexity from minimal promoters to promoters including upstream elements and enhancers. The promoter is typically selected from promoters which are functional in mammalian cells, although prokaryotic promoters and promoters functional in other eukaryotic cells may be used. The promoter is typically derived from promoter sequences of viral or eukaryotic genes. For example, it may be a promoter derived from the genome of a cell in which expression is to occur. With respect to eukaryotic promoters, they may be promoters that function in a ubiquitous manner (such as promoters of a-actin, b-actin, tubulin, human elongation factor1-alpha (EF1-alpha)) or, alternatively, a tissue-specific manner (such as promoters of the genes for pyruvate kinase). Viral promoters may also be used, for example the moloney murine leukaemia virus long terminal repeat (MMLV LTR) promoter, the rous sarcoma virus (RSV) LTR promoter or the human cytomegalovirus (CMV) IE promoter.

In some embodiments, the promoters are inducible so that the levels of expression of the heterologous gene can be regulated during the life-time of the cell. Inducible means that the levels of expression obtained using the promoter can be regulated.

Chimeric promoters may also be used comprising sequence elements from two or more different promoters described above.

In one embodiment, the vectors used in the present invention further comprise an origin of replication.

The vectors of the invention may be used, for example, to transfect or transform a target cell.

The vector comprising a nucleic acid encoding a nucleic acid of the invention is preferably a non-viral vector or a viral vector.

A non-viral vector may be selected in the group consisting of a plasmid, a liposomal nucleic acid complex and a carrier-associated nucleic acid.

By "plasmid", it is herein meant a double-stranded circular DNA. The plasmid may include a marker gene enabling to select the cells comprising said plasmid, an origin of replication to allow the cell to replicate the plasmid and/or a multiple cloning site allowing the insertion of a DNA fragment, in particular the nucleic acid encoding a nucleic acid of the invention.

Non-limitative examples of carrier-associated nucleic acids are polymer-carried DNA and cationic lipids.

The liposomes used in liposomal DNA complexes are well-known in the art. Said liposomes may be cationic, anionic or neutral liposomes.

Cationic lipids are also known in the art and are commonly used for gene delivery. Such lipids include Lipofectin Tm also known as DOTMA (N- [l- (2, 3-dioleyloxy) propyls N, N, N-trimethylammonium chloride), DOTAP (1, 2-bis (oleyloxy)-3 (trimethylammonio) propane), DDAB (dimethyldioctadecyl- ammonium bromide), DOGS (dioctadecylamidologlycyl spermine) and cholesterol derivatives such as DC-Chol (3 beta-(N- (N', N'- dimethyl aminomethane)-carbamoyl) cholesterol). Cationic lipids for gene delivery are preferably used in association with a neutral lipid such as DOPE (dioleyl phosphatidylethanolamine).

By "viral vector", it is herein meant a recombinant viral vector.

The viral vector is preferably selected in the group consisting of a retrovirus vector, a lentivirus vector, Herpes simplex viral vector, an adenovirus vector and an adeno-associated virus vector, preferably a lentivirus vector.

A "retrovirus vector" herein refers to a genetically engineered a retrovirus based vector that cannot replicate and produce progeny infectious virus particles once the virus has entered the target cell. A retrovirus vector genome consists in a single-stranded

positive-sense RNA that encodes a transcriptase enabling to generate double-stranded DNA.

There are many retroviruses that are widely used for delivery of genes both in tissue culture conditions and in living organisms. Examples include and are not limited to murine leukemia virus (MLV), human immunodeficiency virus (HIV-1), equine infectious anaemia virus (EIAV), mouse mammary tumour virus (MMTV), rous sarcoma virus (RSV), fujinami sarcoma virus (FuSV), moloney murine leukemia virus (Mo-MLV), FBR murine osteosarcoma virus (FBR MSV), moloney murine sarcoma virus (Mo-MSV), abelson murine leukemia virus (A-MLV), avian myelocytomatosis virus-29 (MC29), and avian erythroblastosis virus (AEV) and all other retroviridiae including lentiviruses. A detailed list of retroviruses may be found in Coffin *et al.*, 1997, "retroviruses", Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Var us pp 758-763.

The basic structure of a retrovirus genome is a 5' LTR and a 3' LTR, between or within which are located a packaging signal to enable the genome to be packaged, a primer binding site, integration sites to enable integration into a target cell genome and gag, pol and env genes encoding the structural and enzymatic components - these are polypeptides required for the assembly of viral particles. More complex retroviruses have additional features, such as rev and RRE sequences in HIV, which enable the efficient export of RNA transcripts of the integrated provirus from the nucleus to the cytoplasm of an infected target cell.

In the provirus, these genes are flanked at both ends by regions called long terminal repeats (LTRs). The LTRs are responsible for pro viral integration, and transcription. LTRs also serve as enhancer-promoter sequences and can control the expression of the viral genes. Encapsidation of the retroviral RNAs occurs by virtue of a psi sequence located at the 5' end of the viral genome.

The LTRs themselves are identical sequences that can be divided into three elements, which are called U3, R and U5. U3 is derived from the sequence unique to the 3' end of the RNA. R is derived from a sequence repeated at both ends of the RNA and U5 is derived from the sequence unique to the 5' end of the RNA. The sizes of the three elements can vary considerably among different retroviruses.

More preferably, the viral vector is a targeted vector that is it has a tissue tropism which is altered compared to the native virus, so that the vector is targeted to particular cells. This may be achieved by modifying the retroviral Env protein. Preferably the envelope protein is a non-toxic envelope or an envelope which may be produced in non-toxic amounts within the primary target cell, such as for example a MMLV amphotropic envelope or a modified amphotropic envelope.

Preferably the envelope is one which allows transduction of human cells. Examples of suitable env genes include, but are not limited to, VSV-G, a MLV amphotropic env such as the 4070 A env, the RD114 feline leukaemia virus env or haemagglutinin (HA) from an influenza virus. The Env protein may be one which is capable of binding to a receptor on a limited number of human cell types and may be an engineered envelope containing targeting moieties. The env and gag-pol coding sequences are transcribed from a promoter and optionally an enhancer active in the chosen packaging cell line and the transcription unit is terminated by a polyadenylation signal. For example, if the packaging cell is a human cell, a suitable promoter-enhancer combination is that from the human cytomegalovirus major immediate early (hCMV-MIE) gene and a polyadenylation signal from SV40 virus may be used. Other suitable promoters and polyadenylation signals are known in the art.

In one embodiment, the retrovirus vector is a murine leukemia virus (MLV) vector. Retroviral vectors derived from the amphotropic Moloney murine leukemia virus (MLV-A) are commonly used in clinical protocols worldwide.

The "lentivirus vector" is a single-stranded RNA that integrates in the genome of the target cell, thereby allowing long term expression of the nucleic acid. The lentivirus is a genus of viruses of the retroviridae family, characterized by a long incubation period. A distinction between the lentivirus family and other types of retroviruses is that lentiviruses have the capability to infect both dividing and non-dividing cells (Lewis, 1992; Lewis and Emerman, 1994). A detailed list of lentiviruses may be found in Coffin *et al.* ("Retroviruses" 1997 Cold Spring Harbour Laboratory Press Eds: JM Coffin, SM Hughes, HE Varmus pp 758-763). In brief, lentiviruses can be divided into primate and non-primate groups. Examples of primate lentiviruses include but are not limited to: the human immunodeficiency virus (HIV), the causative agent of human acquired-immunodeficiency syndrome (AIDS), and the simian immunodeficiency virus (SIV). The non-primate lentiviral group includes the prototype "slow virus" visna maedi virus (VMV), as well as the related caprine arthritis-encephalitis virus (CAEV), equine infectious anaemia virus (EIAV) and the more recently described feline immunodeficiency virus (FIV) and bovine immunodeficiency virus (BTV). In one embodiment the lentiviral vector is a HIV-based VSV-pseudotyped defective vector.

"Herpes simplex virus" (HSV) is an enveloped, double-stranded (ds) DNA virus. The mature virion consists of different components: an external envelope containing about 13 glycoproteins involved in different functions, among which the first steps of binding and entry into the target cell; an amorphous layer known as the tegument, containing some 20 different proteins with structural and regulatory roles; and an icosadeltahedral capsid

containing a toroidal dsDNA. In one embodiment, the Herpes simplex virus vector is in particular a HSV-1 vector. Different types of HSV-1 vectors a known to the skilled in the art, such as amplicons, replication-defective and replication-competent vectors.

The "amplicons" are plasmid-derived vectors engineered to contain both the origin of HSV DNA replication (*ori*) and HSV cleavage–packaging recognition sequences (*pac*). When amplicons are transfected into mammalian cells with HSV helper functions, they are replicated, form head-to-tail linked concatamers and are then packaged into viral particles. There are two major methods currently used for producing amplicon particles, one based on infection with defective helper HSVs and the other based on transfection of HSV-1 genes, such as a set of *pac*-deleted overlapping cosmids or a pac-deleted and ICP27-deleted BAC-HSV-1.

"Replication-defective vectors" are made of mutant viruses with deletions in one or more genes essential for the lytic cycle, whereas "replication-competent vectors" are composed of attenuated viruses where genes that are not essential for replication in cultured cells *in vitro* are either mutated or deleted (Roberto Manservigi *et al.* 2010, "HSV Recombinant Vectors for Gene Therapy", Open Virol J. 2010; 4: 123–156).

The "adenovirus" is a double-stranded, linear DNA virus that does not go through an RNA intermediate. There are over 50 different human serotypes of adenovirus divided into 6 subgroups based on the genetic sequence homology. The natural target of adenovirus is the respiratory and gastrointestinal epithelia, generally giving rise to only mild symptoms. Serotypes 2 and 5 (with 95% sequence homology) are most commonly used in adenoviral vector systems and are normally associated with upper respiratory tract infections in the young.

Adenoviruses are non-enveloped, regular icosohedrons. A typical adenovirus comprises a 140nm encapsidated DNA virus. The icosahedral symmetry of the virus is composed of 152 capsomeres: 240 hexons and 12 pentons. The core of the particle contains the 36kb linear duplex DNA which is covalently associated at the 5' ends with the Terminal Protein (TP) which acts as a primer for DNA replication. The DNA has inverted terminal repeats (ITR) and the length of these varies with the serotype.

The adenovirus is a double stranded DNA non-enveloped virus that is capable of *in vivo* and *in vitro* transduction of a broad range of cell types of human and non-human origin. These cells include respiratory airway epithelial cells, hepatocytes, muscle cells, cardiac myocytes, synoviocytes, primary mammary epithelial cells and post-mitotically terminally differentiated cells such as neurons.

Adenoviral vectors are also capable of transducing non dividing cells. This is very important for diseases, such as cystic fibrosis, in which the affected cells in the lung

epithelium have a slow turnover rate. In fact, several trials are underway utilizing adenovirus-mediated transfer of cystic fibrosis transporter (CFTR) into the lungs of afflicted adult cystic fibrosis patients.

Adenoviruses have been used as vectors for gene therapy and for expression of heterologous genes. The large (36 kilobase) genome can accommodate up to 8kb of foreign insert DNA and is able to replicate efficiently in complementing cell lines to produce very high titres of up to 10^{12}. Adenovirus is thus one of the best systems to study the expression of genes in primary non-replicative cells.

The expression of viral or foreign genes from the adenovirus genome does not require a replicating cell. Adenoviral vectors enter cells by receptor mediated endocytosis. Once inside the cell, adenovirus vectors rarely integrate into the host chromosome. Instead, it functions episomally (independently from the host genome) as a linear genome in the host nucleus.

An "adeno-associated virus vector", also called AAV is a single-stranded, either positive- or negative-sensed DNA, virus, with an apparent lack of pathogenicity. It infects dividing and non-dividing cells and has the ability to stably integrate into the target cell genome at a specific site (designated AAVS1) in the human chromosome 19. The AAV genome integrates most frequently into the mentioned site, while random incorporations into the genome take place with a negligible frequency. The genome comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs): rep and cap. The former is composed of four overlapping genes encoding Rep proteins required for the AAV life cycle, and the latter contains overlapping nucleotide sequences of capsid proteins: VP1, VP2 and VP3, which interact together to form a capsid of an icosahedral symmetry. Removal of the *rep* and *cap* from the DNA of the vector eliminates the above-mentioned integrative capacity.

In one embodiment, an adeno-associated virus vector is selected from the group consisting of an AAV1 vector, AAV2 vector, AAV6 vector, AAV8 vector, AAV9 vector and a hybrid AAV vector.

A "hybrid AAV vector" is a vector comprising the rep gene of one AAV vector and the cap gene of another AAV vector.

Non-limitative examples of hybrid AAV vector are an AAV1/2 vector, i.e. a vector comprising AAV1 rep gene and AAV2 cap gene, or an AAV2/9 vector, i.e. a vector comprising with AAV2 rep gene and AAV9 cap gene.

An example of AAV2/9 vector is pZac2.1 (for example from Penn Vector Core, Upenn).

By “target cells” is meant herein the MCH expressing neurons, located within the lateral hypothalamus and zona incerta. Despite this restricted distribution MCH neurons project widely throughout the brain.

By "MCH peptide expression activator" is meant herein a natural or synthetic compound that has a biological effect to activate the expression of a nucleic acid encoding an MCH peptide as defined above.

The term "expression" when used in the context of expression of a gene or nucleic acid refers to the conversion of the information, contained in a gene, into a gene product. A gene product can be the direct transcriptional product of a gene (e.g., mRNA, tRNA, rRNA, antisense RNA, ribozyme, structural RNA or any other type of RNA) or a protein produced by translation of a mRNA. Gene products also include messenger RNAs which are modified, by processes such as capping, polyadenylation, methylation, and editing, and proteins (e.g., calpain or calpastatin) modified by, for example, methylation, acetylation, phosphorylation, ubiquitination, SUMOylation, ADP-ribosylation, myristilation, and glycosylation.

Examples of agonists of a MCH receptor are cited for example in MacNeil et al., 2009 and include the peptide of sequence DFDMLRCMLGRVFRPCWQY (SEQ ID NO: 8), wherein the last amino-acid is preferably radiolabeled. Examples of agonists of a MCH receptor also include the peptide of sequence of SEQ ID NO: 6 consisting of the 6–16 segment of MCH, which was demonstrated as being a full agonist of MCHR1 and MCHR2 of potency similar to those of the full-length MCH. Examples of MCHR agonists also include the peptide of sequence SEQ ID NO: 7 which consists of the 6-17 segment of MCH.

Other examples of MCHR agonists include compounds disclosed in EP2098242, WO200808357, US20080124319, US20040242487 and US5449766.

Treatment of ALS

As used herein, the term "Amyotrophic lateral sclerosis" or "ALS" refers to a rapidly progressive, invariably fatal neurological disease affecting motor neurons and nerve cells responsible for controlling voluntary muscles. The disease belongs to a group of disorders known as *motor neuron diseases (MNDs),* which are characterized by the gradual degeneration and death of motor neurons. In 90 to 95 percent of all ALS cases, the disease occurs apparently at random with no clearly associated risk factors. Individuals with this sporadic form of the disease do not have a family history of ALS, and their family members are not considered to be at increased risk for developing it.

About 5 to 10 percent of all ALS cases are inherited. The familial form of ALS usually results from a pattern of inheritance that requires only one parent to carry the gene responsible for the disease. Subsets of ALS cases are of familial origin and five major genes are currently associated with familial ALS (*C9ORF72, SOD1, FUS, TARDBP* and *TBK1*). The *SOD1* gene was the first associated with ALS and most ALS mouse models currently used are based upon overexpression of mutant forms of *SOD1.*

In the context of the invention, ALS can be sporadic ALS or inherited ALS. Preferably, ALS is inherited ALS.

In the context of the invention, inherited ALS can result from at least one mutation in the *C9ORF72, SOD1, FUS, TARDBP* and/or *TBK1* gene. Preferably, inherited ALS results from at least one mutation in the *SOD1* gene.

As used herein, the term "subject" refers to any human or animal subject suffering from ALS.

Preferably," subject" refers to a human.

The present invention concerns an agonist of a MCH receptor, as defined above, for use for reestablishing a metabolic balance in a subject suffering from ALS, as defined above.

The present invention concerns an agonist of a MCH receptor, as defined above, for use in the treatment of ALS for reestablishing a metabolic balance in a subject suffering from ALS, as defined above.

The invention also concerns the use of an agonist of a MCH receptor, as defined above, for the manufacture of a medicament intended for reestablishing a metabolic balance in a subject suffering from ALS, as defined above.

The invention also concerns the use of an agonist of a MCH receptor, as defined above, for the manufacture of a medicament intended for the treatment of ALS for reestablishing a metabolic balance in a subject suffering from ALS, as defined above.

The invention further concerns a method for reestablishing a metabolic balance in a subject suffering from ALS, as defined above, comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor as defined above.

The invention further concerns a method of treatment of ALS for reestablishing a metabolic balance in a subject suffering from ALS, as defined above, comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor as defined above.

By "metabolic balance" is meant herein equilibrium between the intake of nutrients and their eventual loss through absorption or excretion. In a positive balance the intake of a nutrient exceeds its loss; in a negative balance a nutrient is used or excreted faster than it is consumed in the diet. In case of ALS, the metabolic balance is importantly negative as ALS patients are reported to consume 15–16% fewer calories than required to meet their energetic needs, and are thus subject to hypermetabolism.

By "reestablishing a metabolic balance" is meant herein reestablishing an equilibrium between food intakes and expenses in order to establish a metabolic balance corresponding to a healthy subject.

The present invention also concerns an agonist of a MCH receptor, as defined above, for use for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS, as defined above.

The present invention also concerns an agonist of a MCH receptor, as defined above, for use in the treatment of ALS for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS, as defined above.

The invention also concerns the use of an agonist of a MCH receptor, as defined above, for the manufacture of a medicament intended for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS, as defined above.

The invention also concerns the use of an agonist of a MCH receptor, as defined above, for the manufacture of a medicament intended for treating ALS by preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS, as defined above.

The invention further concerns a method for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS, as defined above, comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor as defined above.

The invention further concerns a method of treatment of ALS for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS, as defined above, comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor as defined above.

Preferably, weight loss is prevented, slowed or stopped or weight gain is promoted by the reestablishment of a metabolic balance in said subject.

Preferably, weight loss is prevented, slowed or stopped, so that it is inferior to a loss of 3 to 5 kg. Still preferably, weight loss is prevented, slowed or stopped, so that the BMI of the subject is superior to 18.5 kg/m^2.

The present invention also concerns an agonist of a MCH receptor, as defined above, for use for prolonging life expectancy of a subject suffering from ALS, as defined above.

The present invention also concerns an agonist of a MCH receptor, as defined above, for use in the treatment of ALS for prolonging life expectancy of a subject suffering from ALS, as defined above.

The invention also concerns the use of an agonist of a MCH receptor, as defined above, for the manufacture of a medicament intended for prolonging life expectancy of a subject suffering from ALS, as defined above.

The invention also concerns the use of an agonist of a MCH receptor, as defined above, for the manufacture of a medicament intended for treating ALS by prolonging life expectancy of a subject suffering from ALS, as defined above.

The invention further concerns a method for prolonging life expectancy of a subject suffering from ALS, as defined above, comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor as defined above.

The invention further concerns a method of treatment of ALS for prolonging life expectancy of a subject suffering from ALS, as defined above, comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor as defined above.

Indeed, it was shown in the art, for example in Desport et al., 1999 that the occurrence of malnutrition in patients studied was 16.4%. Survival was worse for malnourished patients, with a 7.7-fold increased risk of death. More recently the same group has shown that a modest weight loss of 5% of initial body weight (or 3-5 kgs) at diagnosis was strongly correlated with very poor prognosis (Marin, 2011). Several more recent studies confirm the observation that weight loss (and / or malnutrition), defined as Body Mass Index BMI ≤ 18.5kg/m2, is an independent, negative, prognostic indicator for survival.

"Prolonging life expectancy" is also referred to and encompasses "long-term survival time" or "reducing mortality or morbidity", which as used herein relates to the superiority of an agonist of a MCH receptor, in particular MCH, to placebo, in particular non-treatment, in subjects suffering from ALS, meaning that the subjects have a higher life span following an administration as described herein, in particular on a regular daily basis. Typically, ALS leads to death within 3 to 5 years after onset in human subjects.

Accordingly, a prolonged life expectancy as intended herein is preferably a lengthening of the life expectancy of at least 5 years.

The present invention also concerns an agonist of a MCH receptor, as defined above, for use in the treatment of ALS, as defined above.

The invention also concerns the use of an agonist of a MCH receptor, as defined above, for the manufacture of a medicament intended for treating a subject suffering from ALS, as defined above.

The invention further concerns a method for the treatment of ALS, as defined above, comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor as defined above.

In the context of the invention, the term "treating" or "treatment" means reversing, alleviating, inhibiting the progress of, or preventing the disorder or condition to which such term applies, or one or more symptoms of such disorder or condition.

Preferably, ALS is treated by preventing, slowing or stopping weight loss or promoting weight gain, in particular by reestablishing a metabolic balance.

By a "therapeutically effective amount" of a compound of the invention is meant a sufficient amount of the compound to treat ALS, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the compounds of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treating and the severity of the disorder, activity of the specific compounds employed, the specific combinations employed, the age, body weight, general health, sex and diet of the subject, the time of administration, route of administration and rate of excretion of the specific compounds employed, the duration of the treatment, drugs used in combination or coincidental with the specific compounds employed, and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compounds at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

Preferably, the agonist of a MCH receptor, in particular the MCH peptide, is administered at a low dose.

By "low dose" is meant herein a dose lower than the one conventionally used to increase food intake. Doses conventionally used to increase food intake in mice are typically 3µg/day (Ito et al., 2003). Preferably, a low dose refers to a dose at least 2 fold lower, more preferably at least 3 fold lower, still preferably at least 4 fold lower, most preferably at least 5 fold lower than the dose conventionally used to increase food intake.

The agonist of a MCH receptor used in the context of the invention may be administered by any suitable route, such as intravenous, intradermal, intracerebroventricular, subcutaneous, intramuscular, intraperitoneal, oral (*e.g.*, buccal, inhalation, nasal and pulmonary spray), intradermal, transdermal (topical), transmucosal or intraocular route.

Preferably, the agonist of a MCH receptor, in particular the MCH peptide, is administered by the intracerebroventricular route.

Preferably, in particular when the pharmaceutical compositions are for parenteral administration, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. Solutions comprising compounds of the invention as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in the appropriate solvent with several of the other ingredients

enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile- filtered solution thereof. The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small tumor area.

The agonist of a MCH receptor may further be used in combination with another anti-ALS therapy.

By "anti-ALS therapy" is meant herein any anti ALS treatment approved for use in a subject in need thereof.

Such anti-ALS therapies are well-known from the skilled person and include mostly glutamate antagonists (Rilutek), which provide protection from amino-acid-derived excitotoxicity intracellular processes, or free radical scavengers (Radicut).

Such therapies do not reverse the course of the disease and are purely supportive. They do not act on the metabolic disorders caused by ALS.

Throughout the instant application, the term “comprising” is to be interpreted as encompassing all specifically mentioned features as well optional, additional, unspecified ones. As used herein, the use of the term “comprising” also discloses the embodiment wherein no features other than the specifically mentioned features are present (*i.e.* “consisting of”). Furthermore the indefinite article "a" or "an" does not exclude a plurality. The mere fact that certain measures are recited in mutually different dependent claims does not indicate that a combination of these measures cannot be used to advantage.

The invention will now be described in more details with reference to the following figures and examples.

Brief description of the sequences

SEQ ID NO: 1 shows the amino acid sequence of full-length MCH peptide.

SEQ ID NO: 2 shows the nucleotide sequence encoding full-length MCH peptide.

SEQ ID NO: 3 shows the amino acid sequence of Human MCHR1.

SEQ ID NO: 4 shows the amino acid sequence of Human MCHR2.

SEQ ID NO:5 shows the amino acid sequence of proMCH.

SEQ ID NO: 6 shows the amino acid sequence of the 6-16 fragment of peptide MCH RCMLGRVYRPC.

SEQ ID NO: 7 shows the amino acid sequence of the 6-17 fragment of peptide MCH RCMLGRVYRPCW.

SEQ ID NO: 8 shows the amino acid sequence of the MCH agonist (prot) DFDMLRCMLGRVFRPCWQY.

See Table 1 for primer sequences identified as SEQ ID NO: 9 to SEQ ID NO: 44.

Brief description of the figures

Figure 1: Effects of pioglitazone on peripheral biomarkers in ALS patients

A: summary of metabolic effects of pioglitazone (and TZDs) in humans

B-E: Changes in plasma adiponectin (% from the baseline) (B), glycaemia (% from the baseline, C), circulating aspartate amino-transferase (ASAT, changes in U/L from the baseline, D), and alanine amino-transferase (ALAT, changes in U/L from the baseline, E). Pioglitazone treated patients are significantly different from placebo treated patients for these items as assessed using a mixed effects regression model analysis. Data are presented as mean and standard error.

Figure 2: No effect of pioglitazone on weight loss in ALS patients

Weight loss (kg loss per month, A, C, E, G) and changes in BMI from the baseline (B, D, F, H) in the whole ALS population (A, B), spinal onset patients (C, D), in patients with relatively preserved quality of life (E, F) and in patients with preserved bulbar function (G, H). To select the patients with preserved quality of life, the inventors used the results from the EuroQoL questionnaire to identify patients that had no or only few problems with their everyday-life. Selected patients answered that they had either no or few problems in their everyday life during at least 6 months after their allocation to a group. To select the patients with preserved bulbar function, the inventors used the results from ALS-FRS-R bulbar subscale and selected patients with a score equal or superior to 10 (maximum: 12) six months after inclusion. No significant difference is noted for these items. Data are presented as mean and standard error.

Figure 3: No effect of piogliotazone on SOD1m mice food intake.

A: Experimental scheme: pioglitazone was provided *per os* after 6 hours of fasting. Food was reintroduced 1 hour after gavage, and food intake recorded for the next 24 hours.

B,C: food intake after pioglitazone treatment in SOD1(G86R) mice (SOD1m) and control littermates (wt) at 75 days of age (n=12, B) and symptom onset (n=13, C), either treated with vehicle ((1) columns) or pioglitazone 40mg/kg body weight ((2) columns).*p< 0.05, Paired Student's t-test for the drug. Data are presented as mean and standard error.

Figure 4: Defect in melanin concentrating hormone (MCH)

A: mRNA levels of hypothalamic neuropeptides in the hypothalamus of SOD1 (G86R) mice (light grey columns, SOD1m) and control littermates (dark grey columns, wt) at 75 days of age (n= 15,a) and symptom onset (n=11, b). POMC: Proopiomelanocortin, AgRP: Agouti Related Peptide, MCH: Melanin concentrating hormone, CART: cocaine- and amphetamine-regulated transcript, NPY: neuropeptide Y, AVP: Arginine vasopressin, CRH: Corticotropin-releasing hormone, TRH: Thyrotropin-releasing hormone, SST: somatostatin, ORX: orexin, NTS: neurotensin.

B: Quantification of MCH neurons in the lateral hypothalamus (LH). MCH immunohistochemistry was performed on half of whole brain sections, and lateral hypothalamus was identified according to Paxinos Brain Atlas (scheme of identified regions, in black, a). Representative images are shown for SOD1(G86R) mice and control littermates at 75 days of age at two magnifications (scale bar 200 µm (middle row), 20µm (lower row)). Total numbers of MCH positive cell bodies in the lateral hypothalamus were determined in SOD1m mice at 75 days of age (n=8, b-f) and at symptom onset (n=7, b) as compared with their wt littermates.

C: Dosing of MCH in Cerebrospinal Fluid (CSF) of control patients (left column, n=9) and ALS patients (right column, n=18).

Figure 5: Multiple ALS mouse models display functional and molecular alterations in hypothalamic melanocortin system

A-B: Food intake was measured during one hour, after either seven hours (A) or one hour (B) of fasting in SOD1(G86R) mice (black columns, SOD1m) and control littermates (white columns, wt) at 75 days of age (n= 10 and n=14 respectively for A and B). *p<0.05, Student's t-test. Data are presented as mean and standard error.

C: mRNA levels of POMC and AgRP in the hypothalamus of transgenic mice expressing A315T TDP-43 mutation (black columns, TDP43m) and control littermates (white columns, wt) at non symptomatic stage (n=6). Unpaired t-test. *p<0.05.

D: Food intake was measured one hour after refeeding in TDP43m mice (n=8). *p<0.05, **p<0.01, Multiple t-test. Data are presented as mean and standard error.

E: mRNA levels of POMC and AgRP in the hypothalamus of transgenic mice *Fus* ΔNLS/+ knock-in mice) (black columns, ΔNLS/+) and control littermates (white columns, +/+) at 10 months of age (n=4). Data are presented as mean and standard error.

F: Food intake was measured one hour after refeeding in ΔNLS/+ mice (n=10) at 10 months of age. *p<0.05, Student's t-test. Data are presented as mean and standard error.

Figure 6: Hypothalamic pathological alterations of lateral hypothalamus

A: pTDP43 pathological staining in the lateral hypothalamus of post-mortem patient tissues. Representative images are shown for ALS patients (a,b,c, scale bar 20µm). pTDP43 staining in the lateral hypothalamus (LH) and Arcuate Nucleus (Arc) were scored by a blind observer in ALS post mortem patient tissues as compared with their control patient tissues (d, n=10).

B: p62 pathological aggregates in the lateral hypothalamus of mice. p62 immunohistochemistry was performed on selected sections after identification of lateral hypothalamus according to Paxinos Brain Atlas (identified regions in box of a and b). Representative images are shown for SOD1 (G86R) (b,d,e) mice and control littermates (a,c) at onset of symptoms at three magnifications (scale bar 1000 µm (upper row), 250µm (middle row), 50µm (lower row)). p62 aggregates in the lateral hypothalamus (LH), cortex and hippocampus were scored by a blind observer in SOD1m mice ((b) columns) at onset of symptoms as compared with their wt littermates ((a) columns) (f, n=4).

Figure 7: MCH injection increase weight gain in SOD1 mice

A: Weight gain evolution of SOD1(G86R) mice (SOD1m) at 75 days of age at surgery, after Intra Cerebro Ventricular injection (ICV) and constant diffusion of MCH (1.2µg/day, (2) line) or vehicle ((1) line) with an osmotic pump (n=13). B: Weight gain evolution of wt littermate mice (wt) at 75 days of age at surgery, after Intra Cerebro Ventricular injection (ICV) and constant diffusion of MCH (1.2µg/day, (2) line) or vehicle ((1) line) with an osmotic pump (n=16).

C: Factor of weight gain from linear regression of previous data for each SOD1m mice(A) and each wt mice (B) during the 13 days of diffusion in the lateral ventricle of MCH (1.2µg/day, (2) columns) or vehicle ((1) columns) with an osmotic pump (n=13 for SOD1m and n=16 for wt).

Figure 8: MCH injection does not increase food intake in SOD1 mice

Cumulative food intake over 14 days for mice presented in Figures 7A and 7B.
Data are presented as mean and standard error.

Examples

EXAMPLE 1: Alterations in the hypothalamic melanocortin pathway in amyotrophic lateral sclerosis

Materials and Methods

Patients and treatments

All the biological materials from human ALS patients were sampled as part of the GERP-ALS trial (clinicaltrials.gov reference: NCT00690118). Briefly, patients with possible, probable (clinically or laboratory-supported) or definite ALS according to the revised version of the El Escorial criteria were considered for enrolment into the study. Included patients displayed onset of progressive weakness within 36 months prior to study and had a disease duration of more than six months and less than three years (inclusive) with disease onset defined as date of first muscle weakness, excluding fasciculation and cramps. They reached a best-sitting slow vital capacity between 50 % and 95 % of predicted normal. They were capable of thoroughly understanding the information provided and gave written informed consent. All included patients had been treated with 100 mg riluzole daily for at least three months prior to inclusion. Detailed exclusion and inclusion criteria have been described earlier (Dupuis *et al.*, 2012). The study protocol was approved by the ethics committee of the university of Ulm and all other participating centres.

The two treatment groups were 100 mg riluzole plus 45 mg pioglitazone (pioglitazone group) and 100 mg riluzole plus placebo (placebo group). Patients were randomly assigned to one of the two treatment groups and both groups were matched for age, gender and site of onset (Dupuis *et al.*, 2012).

Procedures and biochemical analysis of human samples

After inclusion, patients underwent a screening phase and a treatment phase (18 months), with stepwise increase in dosage (Dupuis *et al.*, 2012). Clinical and physical examinations, blood sampling, and drug compliance were recorded at on-site visits (1, 2, 6, 12 and 18 months after baseline visit). Body weight was recorded at on-site visits, except for 3, 9 and 15 months timepoints (telephone contacts). There were no differences in results when excluding these three time points. Routine clinical laboratory tests were performed at each on-site visit (baseline and 1, 2, 6, 12 and 18months after baseline). All tests were carried out according to standard laboratory procedures at each study centre's

locally accredited laboratory, which defined the normal reference range for each analyte. The following laboratory tests were performed using standard methods: Alanine Aminotransferase (ALAT), Aspartate Aminotransferase (ASAT), fasting blood glucose. Adiponectin measurements were done in the neurochemical laboratory in Ulm (MSD assay).

Animals

Transgenic mice were housed in the animal facility of the medicine faculty of Strasbourg University, with 12 h/12 h of light/dark and unrestricted access to food and water. In all experiments, littermates were used for comparison. Transgenic SOD1(G86R) were maintained in their initial FVB/N genetic background according to previous studies (Dentel et al, 2013). Transgenic mice expressing TDP43(A315T) were previously described and were maintained as heterozygous in their initial C57Bl6/J background (Wegorzewska et al, 2009). Heterozygous *Fus*$^{\Delta NLS/+}$ are knock-in mice expressing FUS protein deleted from its C-terminal NLS from one of the endogenous *Fus* gene. These mice were generated and maintained in C57Bl6/J background. Tph2-YFP mice were purchased from Jackson laboratories (Bar Harbor, ME, USA; strain 014555) and maintained in their initial genetic background. Female Tph2-YFP mice were crossed with male SOD1(G86R) to generate compound transgenic mice.

For biochemical analysis, animals were sacrificed at the ages indicated at 2PM, and tissues were quickly dissected, frozen in liquid nitrogen, and stored at −80 °C until use. For histological analysis, animals were anesthetized by intraperitoneal injection of ketamine (Imalgène 1000®, Merial, Lyon France; 90 mg/kg body weight) and xylazine (Rompun 2% ®, Bayer, Leverkusen, Germany; 10 mg/kg body weight) at the ages indicated at 2PM. After perfusion of 4% paraformaldehyde (v/v PFA, Sigma, St Louis, MO, USA), brains were removed, stored in the same fixative overnight at 4°C and stored in phosphate buffered saline (PBS) until used. These experiments were authorized by the local ethical committee of Strasbourg University (CREMEAS).

Drugs and treatments

Pioglitazone (Actos®, Takeda, London, UK) was dissolved in 10% (v/v) dimethyl sulfoxide (DMSO, Fisher Scientific, Illkirch, France) and a single oral administration was given by gavage at a dose of 40 mg/kg body weight.

MCH (Sigma, St Louis, MO, USA) was administrated by IntraCerebral Injection (ICV) using continous delivery through Alzet osmotic mini-pumps (see below).

Measurements of food intake

For the pioglitazone experiment, mice were fasted from 9AM to 3PM, and pioglitazone was administrated at 3PM. Food was reintroduced 1 hour after gavage and food intake was recorded during 24h.

For short term fasting experiments, mice were fasted from 8AM to 3PM (7 hours fasting conditions) or from 2PM to 3PM (1 hour fasting conditions) and food was reintroduced after 7 hours or 1 hour of fasting. Food intake was measured one hour and 24 hours after refeeding.

ICV Surgical procedure

75 days old-mice were anesthetized with ketamine and xylazine (respectively 90mg/kg from Imalgene 1000, Merial, Lyon, France and 10mg/kg from Rompun 2%, Bayer, Puteaux, France).Prior to the surgery, 50µL of lidocaine (21.33mg/mL, Xylovet, Ceva, Libourne, France) was injected under the skin at the place of the head skin incision. A sterile brain infusion cannula of Brain Infusion Kit 3 (28-gauge; Alzet, Palo Alto, CA) was stereotaxically implanted into the left lateral ventricle. When a flat skull position was used, the stereotaxic coordinates were 0.4 mm posterior to the bregma, 0.8 mm left lateral to the midline, and 2.0 mm from the surface of the skull. The cannulas were fixed to the skull with instant adhesive gel (loctite 454). The infusion cannula was connected to an osmotic minipump (Alzet, model no. 2002) that was pre-filled with MCH (0.1mg/mL) or vehicle (aLCR,,119mM NaCl, 26.2mM $NaHCO_3$, 2.5mM KCl, 1mM NaH2PO4, 1.3mM $MgCl_2$, 10mM glucose, 2.5mM $CaCl_2$) and had polyvinyl chloride tubing. The mini-pump was then implanted under the skin of the back, and care was taken to clean wound properly with antiseptics. Prior to the implantation, pumps and Brain Infusion Kit were prepared at a depth of 2.0mm and according manufacter's recommendation especially with overnight incubation at 37°C in 0.9% saline solution.

ICV experimental design

Body weight and daily food intake were measured daily between 11am and 1pm during 14 days.

Mouse section histology

Fixed brains were included in 6% (w/v) agar (Sigma, St Louis, MO, USA) and sectioned from Bregma 0.02mm to Bregma -2.90mm into 40µm coronal sections on a vibratome. Lateral hypothalamus was identified according to Paxinos Brain Atlas. POMC and MCH immunohistochemistries were performed on half of selected brain sections. p62

immunohistochemistry was performed on anatomically matched sections. Immunohistochemistry was performed on floating sections using standard histological techniques. Endogenous peroxidases were inactivated using 3% (v/v) H_2O_2. For POMC, MCH and p62 immunohistochemistries, permeabilization and saturation of nonspecific sites were done with 0.25%(v/v) Triton (Sigma, St Louis, MO, USA) and 50mg/ml Bovine Albumin Serum (BSA, Sigma, St Louis, MO, USA). Rabbit anti-POMC primary antibody (Phoenix Peptide, Burlingame, CA, USA; 1:2000), rabbit anti-MCH primary antibody (Phoenix Peptide, Burlingame, CA, USA; 1:2000) or mouse anti-p62(anti sqstm1) primary antibody (Abcam, Cambridge, UK, 1:500) were incubated overnight at room temperature. Biotinylated donkey anti-Rabbit secondary antibody (Jackson, West Grove, PA, USA; 1:500) and biotinylated donkey anti-Mouse secondary antibody (Jackson, West Grove, PA, USA, 1:500) were incubated during 90 minutes at room temperature. Staining was performed using Vectastain Elite ABC kit (Vector, Burlingame, CA, USA). After revelation with 3,3'-Diaminobenzidine (DAB, Sigma, St Louis, MO, USA; 0.5mg/ml), sections were mounted and images of all sections were taken.

For quantification, Bright-field images of lower brain part for POMC and MCH stainings or bright-field images of right Lateral Hypothalamus part for p62 staining were acquired with a Nikon DS –Ri1 camera attached to a Nikon microscope (Nikon Eclipse E800) fitted with respectively a Plan Apo 4x lens (N.A.=0.20, marque) and a plan Apo 10x lens (N.A.=0.45, marque). White balance, gain, exposure, and light settings were kept the same when acquiring all images of a given staining.

Human section histology

Paraffin included human hypothalamus were sectioned in 70µm sections according to the method of Feldengut (2013, J. of Neurosciences Methods).

Every 10 cuts, after deparaffination, free-floating sections were processed immunohistochemically. Endogenous peroxidases were inactivated using 3% (v/v) H_2O_2. Antigen retrieval with performic acid buffer was done in steamer during 30 minutes before permeabilization and saturation of nonspecific sites with 0.25%(v/v) Triton (Sigma, St Louis, MO, USA) and 50mg/ml Bovine Albumin Serum (BSA, Sigma, St Louis, MO, USA). Rabbit anti-pTDP43 (kindly provided by Pr Braak, 1:10000) primary antibody was incubated overnight at room temperature. Biotinylated donkey anti-Rabbit secondary antibody (1:200) and was incubated during 90 minutes at room temperature. Staining was performed using Vectastain Elite ABC kit (Vector, Burlingame, CA, USA). After revelation with 3,3'-Diaminobenzidine (DAB, Sigma, St Louis, MO, USA; 0.5mg/ml), sections were dehydrated and mounted according to Feldengut (2013, J. of Neurosciences Methods).

Image analysis

An operator blinded to the genotype quantified all experiments. Total numbers of POMC positive cell bodies in the arcuate nucleus were determined for each animal and normalized per section. Quantification of p62 and pTDP43 staining was scored by a blinded observer,using a semi-quantitative scale.

RNA extraction and quantitative RT-PCR

RNA was extracted from mouse hypothalami using TRIZOL reagent (Life Technologies, Carlsbad, CA, USA). After reverse transcription with iScript reverse transcription Supermix for RT-qPCR (BioRAD, Hercules, CA, USA), cDNA was obtained from 1 µg of RNA. mRNA levels were obtained by quantitative PCR using Sso Advanced Universal SYBR Green Supermix (BioRAD, Hercules, CA, USA) with corresponding sense and antisense primers. Standard curves were constructed by amplifying serial dilutions of cDNA. Starting quantities of samples were calculated with Biorad software. mRNA levels of genes of interest were normalized to expression levels of the 18S ribosomal, TBP and pol2 RNA housekeeping genes using GeNorm (Vandesompele *et al.*, 2002). Primer sequences are shown in Table 1.

Table 1: Primers used for RT-qPCR

Target	SEQ ID	Forward primer	SEQ ID	Reverse primer
18s	**9**	F-TCTGATAAATGCACGCATCC	**10**	R-GCCATGCATGTCTAAGTACGC
AgRP	**11**	F-CAGGCTCTGTTCCCAGAGTT	**12**	R- TCTAGCACCTCCGCCAAA
AVP	**13**	F-TCTGACATGGAGCTGAGACAG	**14**	R- GAAGCAGCCCAGCTCGT
BDNF	**15**	F- GCCTTTGGAGCCTCCTCTAC	**16**	R- GCGGCATCCAGGTAATTTT
CART	**17**	F-CGAGAAGAAGTACGGCCAAG	**18**	R- CTGGCCCCTTTCCTCACT
CRH	**19**	F-GAGGCATCCTGAGAGAAGTCC	**20**	R- TGTTAGGGGCGCTCTCTTC
Galanin	**21**	F- AGAAGAGAGGTTGGACCCTGA	**22**	R- GAGGCCATGCTTGTCGCTAA
MCH	**23**	F-GCAGAAAGATCCGTTGTCGC	**24**	R-CGGATCCTTTCAGAGCGAGG
NPY	**25**	F- CCGCTCTGCGACACTACAT	**26**	R- TGTCTCAGGGCTGGATCTCT
NTS	**27**	F-AGCCCTGGAGGCAGATCTAT	**28**	R-CCAAGACGGAGGACTTGCTT
Orexin	**29**	F-CTTCAGGCCAACGGTAACCA	**30**	R-GGTGCTAAAGCGGTGGTAGT
PC1	**31**	F- GCTGGTGTGTCTCTGATCTTG	**32**	R- GAGTCCAACCTCTTTGCTCCA
PC2	**33**	F- AAAATACCACCCACCGGCAA	**34**	R- CCAGGTAGCGGACGAAGTTT
POMC	**35**	F- AGTGCCAGGACCTCACCA	**36**	R- CAGCGAGAGGTCGAGTTTG
POL2	**37**	F-GCTGGGAGACATAGCACCA	**38**	R-TTACTCCCCTGCATGGTCTC
SST	**39**	F- GGGCATCATTCTCTGTCTGG	**40**	R- GGGCATCATTCTCTGTCTGG
TBP	**41**	F-CCAATGACTCCTATGACCCCTA	**42**	R-CAGCCAAGATTCACGGTAGAT

TRH	43	F- TGCAGAGTCTCCACCTTGC	44	R- GGGGATACCAGTTAGCACGA

Statistical analysis

For statistical analyses in ALS patients, group comparisons were performed using mixed effects regression model analysis by the Institute of Epidemiology and Medical Biometrics at the University of Ulm using the statistical software package SAS Version 9.2 under Windows.

For animal experiments, comparison of two groups was performed using unpaired Student's t-test, except for experiments with pioglitazone in which paired t-test was used. Comparison of three or four groups was performed using One-way ANOVA and Tukey *post-hoc* test. Statistics in animal experiments were performed using Prism version 6.0.

All results from analysis were regarded as hypothesis generating only. All statistical tests were carried out two-sided at a significance level of 5%.

Results

Normal peripheral response to pioglitazone in ALS patients

The inventors took advantage of data collected during the pioglitazone GERP-ALS trial to investigate energy metabolism in ALS patients in response to pioglitazone (Dupuis et al., 2012).

In this clinical trial, 219 ALS patients had been enrolled, and randomly allocated to either placebo (n=110; bulbar: n=33, spinal: n=77), or pioglitazone (n=109; bulbar: n=32, spinal: n=77) treatment after stratification based on site of onset (bulbar or spinal)(Dupuis *et al.*, 2012).

The metabolic effects of thiazolinediones (TZDs), including pioglitazone are well understood in models and their effects are largely described in patients (Fig. 1 A, Table 2). Pioglitazone treatment is known to increase levels of adiponectin, an adipose-derived hormone through direct transcriptional activation of the adiponectin gene in adipocytes (Maeda *et al.*, 2001). Consistently, pioglitazone treatment multiplied by four the levels of circulating adiponectin in ALS patients after six months of treatment, and this was maintained after 12 months of treatment (Fig. 1 B). Pioglitazone decreases glycemia through hepatic and skeletal muscle PPARγ. In ALS patients, pioglitazone decreased glycaemia (Fig. 1 C), although this effect was milder than observed in other populations, including non-diabetic patients (Belfort *et al.*, 2006). Consistent with a direct action on liver, pioglitazone decreased levels of ASAT and more robustly levels of ALAT (Fig. 1 D-E). In all, pioglitazone displayed the expected metabolic effects on adipocytic, muscular, and hepatic biomarkers, and was likely able to activate PPARγ in these tissues.

Table 2: Numbers of patients for Figure 1

	0	1	2	6	12	18
Adiponectin						
Placebo	58			58	38	
Pioglitazone	27			56	35	
Glycemia						
Placebo	100	84	90	70	47	41
Pioglitazone	104	89	95	79	44	44
ASAT						
Placebo	104	92	93	76	46	43
Pioglitazone	106	98	96	79	48	47
ALAT						
Placebo	104	92	93	76	46	44
Pioglitazone	107	99	97	80	48	47

Pioglitazone does not lead to weight gain in ALS patients

TZDs are also known to act in hypothalamic melanocortin neurons to promote feeding (Diano *et al.*, 2011, Long *et al.*, 2014), and this activation of PPARγ in melanocortin neurons is responsible for the robust weight gain associated with TZDs treatment (Lu *et al.*, 2011, Ryan *et al.*, 2011, Long *et al.*, 2014). Thus, the evolution of body weight upon pioglitazone treatment represents a reliable proximal marker of PPARγ action in hypothalamic melanocortin neurons. In ALS patients, pioglitazone had no effect on weight loss (Fig. 2 A), or BMI (Fig. 2 B, Supplementary Table 2). Increased weight in response to pioglitazone is due to increased food intake, and a subset of ALS patients experience dysphagia. However, pioglitazone had also no effect on BMI and weight loss when considering spinal onset patients (Fig. 2 C-D), or patients with preserved everyday life during at least 6 months (results from EuroQoL EQ-5D questionnaire, Fig. 2 E-F). Last, patients with preserved bulbar function during at least 6 months after inclusion (as assessed using ALS-FRS R bulbar subscale) did not lose weight, yet pioglitazone had no effect on their BMI (Fig. 2 G-H). Importantly, there were no differences among groups for intake of drugs affecting body weight and food intake, in particular anti-epileptics, anti-diabetics, anti-psychotics or SSRIs (Supplementary Table 4). Thus, pioglitazone did not increase weight in ALS patients, and this was not related with dysphagia nor was confounded by intake of other drugs.

Pioglitazone does not increase food intake in mutant SOD1 mice

The inventors hypothesized that the lack of weight gain for ALS patients under pioglitazone was due to defects in stimulating food intake. To test this hypothesis, the inventors turned to transgenic mice expressing mutant SOD1(G86R) (SOD1m mice) as a

model of ALS and set up an experimental protocol to study food intake in response to pioglitazone. In rodents, pioglitazone has various effects on food intake depending on genetic background, dose, route and associated diet (Diano *et al.*, 2011, Ryan *et al.*, 2011, Long *et al.*, 2014). Using a protocol (Fig. 3 A) adapted from Ryan and collaborators (Ryan *et al.*, 2011), the inventors observed that a single oral dose of pioglitazone (40mg/kg) increased food intake by 10-15% in wild type FVB/N mice (Fig. 3 B-C). However, food intake was not increased in littermate SOD1m mice either one month before motor symptoms (Fig. 3 B) or at disease onset (Fig. 3C). Thus, pioglitazone was not able to increase food intake in SOD1m mice.

Defects in melanocortin neurons in mutant SOD1 mice

Hypothalamic melanocortin neurons constitute the primary target of TZDs to promote food intake (Diano *et al.*, 2011, Long *et al.*, 2014). The melanocortin system is mainly constituted of two antagonistic neuronal types located in the arcuate nucleus: pro-opiomelanocortin (POMC) neurons, that secrete the anorexigenic peptide αMSH, and AgRP neurons, that promote food intake, mostly through production of AgRP, an endogenous αMSH antagonist. POMC mRNA levels were two-fold lower in SOD1m mice, either at 75 days of age (Fig. 4 A a.) or at onset (Fig. 4 A b.), while AgRP mRNA levels were higher in non-symptomatic mice, but not at onset (Fig. 4 A a.b.). This involvement of the melanocortin system was relatively selective, as the inventors did not observe expression changes in multiple neuropeptides involved in energy homeostasis, in particular CART, NPY, CRF, AVP, TRH, galanin, somatostatin (SST) and BDNF. Importantly however, the inventors observed significant decreased expression of MCH at both ages (Fig. 4 A a.). Consistent with decreased POMC expression, the inventors observed about 30% less POMC-positive neurons in the arcuate nucleus of SOD1m mice as compared with their wild type littermates at 75 days of age and almost 50% less at onset. In all, the melanocortin system appears shifted towards decreased melanocortin tone in SOD1m mice.

Multiple ALS mouse models display functional and molecular alterations in hypothalamic melanocortin system

The inventors then asked whether the observed melanocortin defects translated into functional abnormalities. Indeed, the combination of decreased POMC with increased AgRP is usually found in situations of promotion of food intake, such as during fasting or in case of leptin deficiency (Mizuno *et al.*, 1998, Mizuno *et al.*, 1999, Ziotopoulou *et al.*, 2000), and compromised melanocortin system is likely to affect food intake behaviour, in

particular during refeeding (Perez-Tilve *et al.*, 2010). Thus, the inventors measured food intake after short term fasting in SOD1m mice. Consistent with decreased POMC levels, one hour food intake of SOD1m mice was two fold higher than wild type littermates after seven hours (Fig. 5 A) or one hour (Fig. 5 B) of fasting. Mutations in SOD1 only account for 20% of familial ALS cases, and alterations in hypothalamic melanocortin pathways could be SOD1-specific. However, POMC mRNA levels were decreased in transgenic mice expressing A315T mutant TDP-43 (Wegorzewska *et al.*, 2009) (Fig. 5 C), and these mice also displayed transient hyperphagia in response to fasting (Fig. 5 D). Last, POMC mRNA levels tended to be lower in 10 months old knock-in mice expressing a truncated FUS protein retained in the cytoplasm ($Fus^{\Delta NLS/+}$ mice) (Fig. 5 E) and $Fus^{\Delta NLS/+}$ mice displayed increased food intake after short term fasting as compared with their littermates (Fig. 5 F). Thus, abnormal food intake behaviour and defects in the melanocortin system is a hallmark of ALS mouse models.

EXAMPLE 2: Identification of the role of MCH in the treatment of ALS

Hypothalamic pathological alterations of lateral hypothalamus

In a first phase, the inventors systematically stained hypothalamic sections of ALS patients (n=10) and controls (n=10) for phosphorylated TDP43 (pTDP43), the major component of ubiquitin positive inclusions in ALS. TDP43 is the classic marker of protein aggregates in ALS. Interestingly, the inventors observed strong accumulations of pTDP43 positive inclusions in the lateral hypothalamic (LH) area, while the arcuate nucleus, another key metabolic nucleus, was roughly devoid of pathological inclusions (Figure 6A). Mice expressing mutant SOD1 do not develop TDP-43 positive inclusions, but rather inclusions positive for p62, a ubiquitin binding protein that accumulates upon UPS or autophagic dysfunction. Consistent with results in ALS patients, the inventors observed many p62 positive aggregates in the LH of mutant SOD1 mice. Thus, the LH area appears involved in ALS, both in models and patients. The presence of protein aggregates demonstrates that this region is specifically affected by disease pathogenesis.(Figure 6B).

Identification of LH affected neurons

The LH is known to express two major neuropeptides, orexin and MCH, two orexigenic peptides. The inventors observed that MCH, but not orexin expression was downregulated in mutant SOD1 mice (Figure 4A). The decrease of MCH expression was also observed

using immunohistochemistry, with decreased numbers of MCH-positive neurons, and overall decreased staining (Figure 4B).

The inventors also observed a decrease of MCH levels in the Cerebrospinal Fluid (CSF) of ALS patients (n=18) in comparison with controls (n=9) (Figure 4C).

Functional role of hypothalamic neuropeptide MCH in weight loss

The inventors then studied whether the loss of MCH could be responsible for weight loss in mutant SOD1 mice. To this aim, they implanted mutant SOD1(G86R) mice at 75 days of age with osmotic mini-pumps either filled with vehicle (artificial CSF,aCSF)(n=13) or MCH (n=13). Control littermates were implanted in parallel (n=16 for vehicle and n=16 for MCH). SOD1(G86R) are already losing weight at 75 days and they are living between 105 and 115 days generally. MCH delivery was at a constant flow of 1,2µg/day during 13 days intra-cerebro ventricularly through a cannula stereotactically inserted in the lateral ventricle. This dose was 3 fold lower than doses known to increase food intake. Importantly, MCH ICV administration led to sustained increased weight gain in mutant SOD1 mice, but not, at that low dose, in wild type controls (figure 7A-B). Indeed, daily weight gain of MCH-treated SOD1m mice was similar to the weight gain of wild type mice with no administration of MCH (Figure 7C). In all, MCH complementation is sufficient to revert weight loss in ALS mice. Importantly, while SOD1 mice treated with MCH gained weight, food intake increase was not observed (Figure 8). This observation tends to point out that MCH is acting on metabolism rather than food intake at those low doses.

This *in vivo* experiment provided the proof of concept that MCH loss was contributing to weight loss.

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CLAIMS

1. Agonist of a Melanin Concentrating Hormone (MCH) receptor for use in the treatment of amyotrophic lateral sclerosis (ALS) for reestablishing a metabolic balance in a subject suffering from amyotrophic lateral sclerosis (ALS).

2. Agonist of a MCH receptor for use in the treatment of ALS for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS.

3. The agonist of a MCH receptor for its use according to claim 2, wherein weight loss is prevented, slowed or stopped or weight gain is promoted by the reestablishment of a metabolic balance in said subject.

4. The agonist of a MCH receptor for its use according to claim 2 or 3, wherein food intake is not increased in said subject.

5. Agonist of a MCH receptor for use in the treatment of ALS for prolonging life expectancy of a subject suffering from ALS.

6. Agonist of a MCH receptor for use in the treatment of ALS.

7. The agonist of a MCH receptor for its use according to any one of claims 1 to 6, wherein said MCH receptor is MCHR1 or MCHR2.

8. The agonist of a MCH receptor for its use according to any one of claims 1 to 7, wherein said agonist is selected from the group consisting of a MCH peptide or a derivative thereof which retains the ability to activate a MCH receptor, small organic molecules, a nucleic acid encoding a MCH peptide or a derivate thereof which retains the ability to activate a MCH receptor and a MCH peptide expression activator.

9. The agonist of a MCH receptor for its use according to claim 8, wherein said agonist is a MCH peptide or a derivative thereof which retains the ability to activate a MCH receptor.

10. The agonist of a MCH receptor for its use according to claim 9, wherein said agonist is the MCH peptide of sequence DFDMLRCMLGRVYRPCWQV (SEQ ID NO: 1).

11. The agonist of a MCH receptor for its use according to any one of claims 1 to 10, wherein said agonist is administered by the intracerebroventricular route.

12. The agonist of a MCH receptor for its use according to any one of claims 1 to 11, for use in combination with another anti-ALS therapy.

13. Method for reestablishing a metabolic balance in a subject suffering from ALS comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor.

14. Method for preventing, slowing or stopping weight loss or promoting weight gain in a subject suffering from ALS comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor.

15. Method for prolonging life expectancy in a subject suffering from ALS comprising the step of administering to said subject a therapeutically effective amount of an agonist of a MCH receptor.

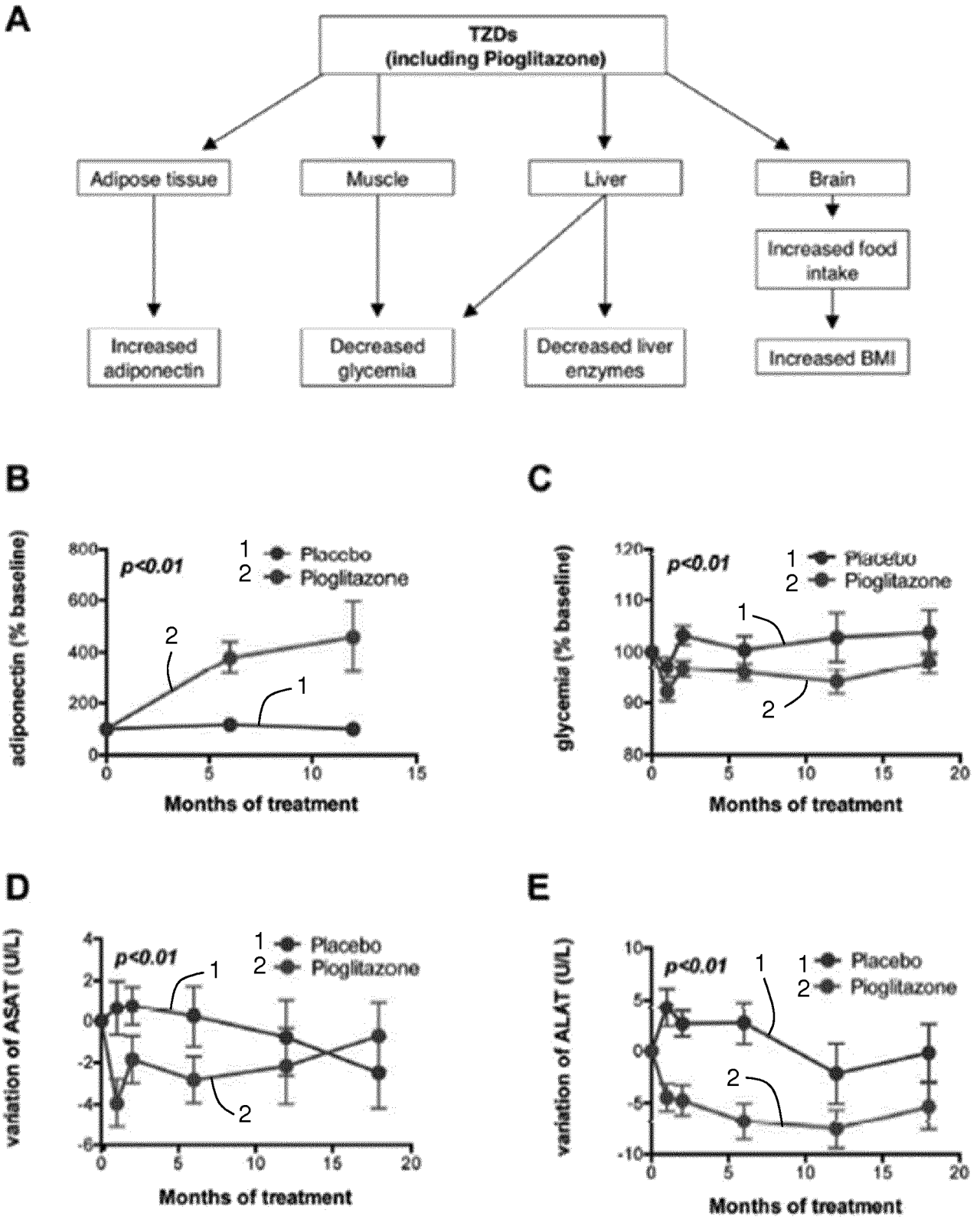

A
TZDs
(including Pioglitazone)
Adipose tissue
Muscle
Liver
Brain
Increased food intake
Increased adiponectin
Decreased glycemia
Decreased liver enzymes
Increased BMI
B
adiponectin (% baseline)
p<0.01
1 Placebo
2 Pioglitazone
Months of treatment
C
glycemia (% baseline)
p<0.01
1 Placebo
2 Pioglitazone
Months of treatment
D
variation of ASAT (U/L)
p<0.01
1 Placebo
2 Pioglitazone
Months of treatment
E
variation of ALAT (U/L)
p<0.01
1 Placebo
2 Pioglitazone
Months of treatment

FIG.1

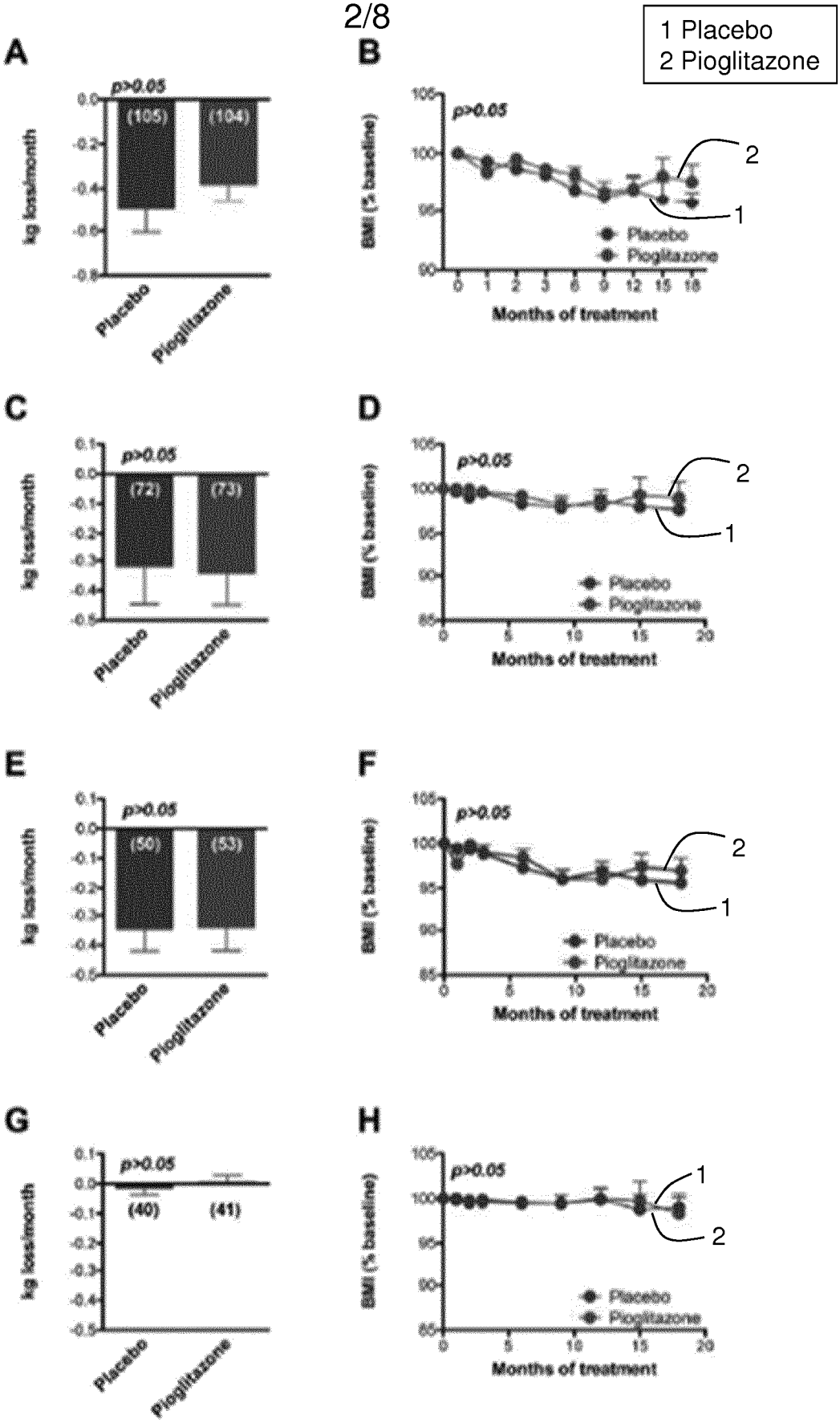
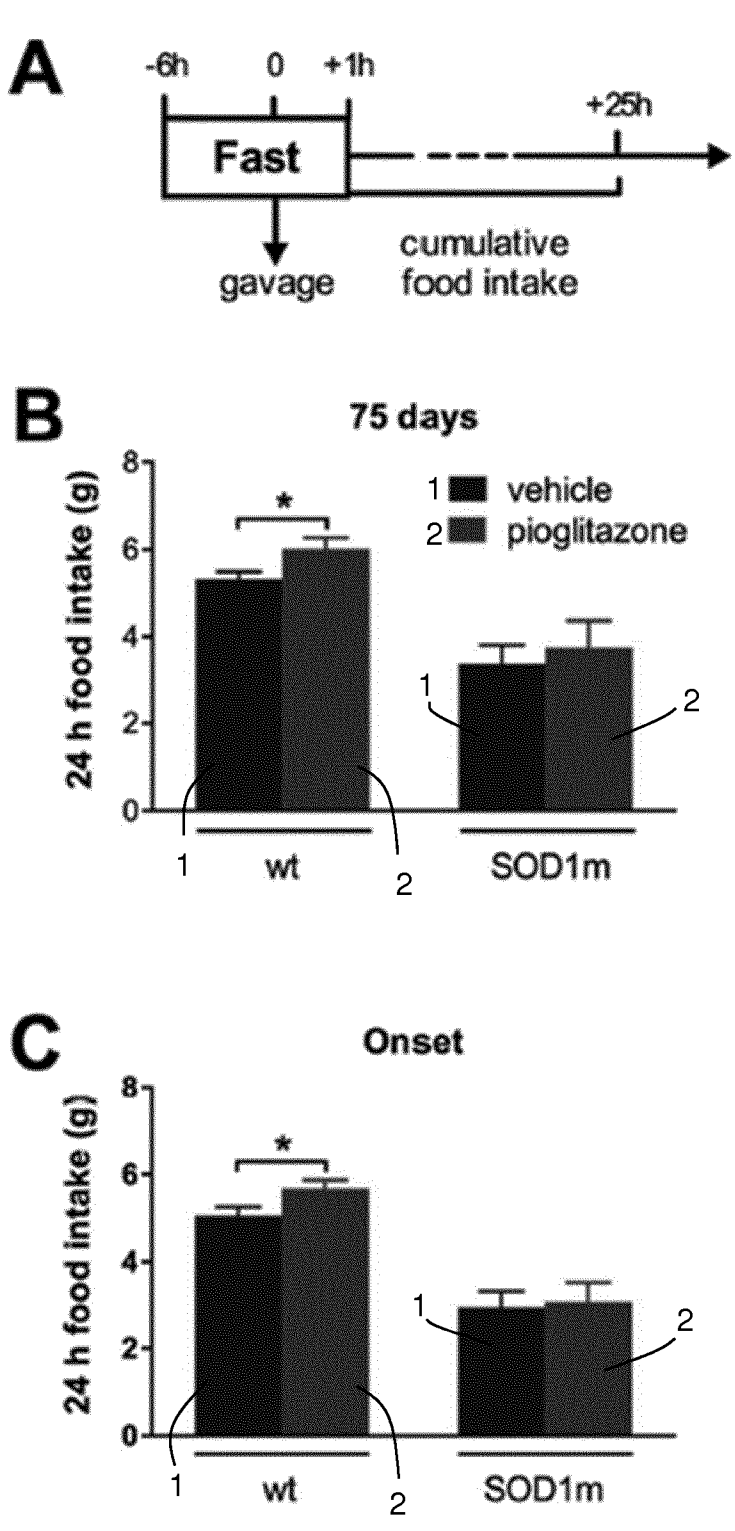

1 Placebo
2 Pioglitazone
A
B
C
D
E
F
G
H
p>0.05
(105)
(104)
(72)
(73)
(50)
(53)
(40)
(41)
kg lost/month
BMI (% baseline)
Placebo
Pioglitazone
Months of treatment

FIG.2

FIG.3


A
-6h
0
+1h
+25h
Fast
gavage
cumulative
food intake
B
75 days
24 h food intake (g)
1 vehicle
2 pioglitazone
*
wt
SOD1m
C
Onset
24 h food intake (g)
*
wt
SOD1m

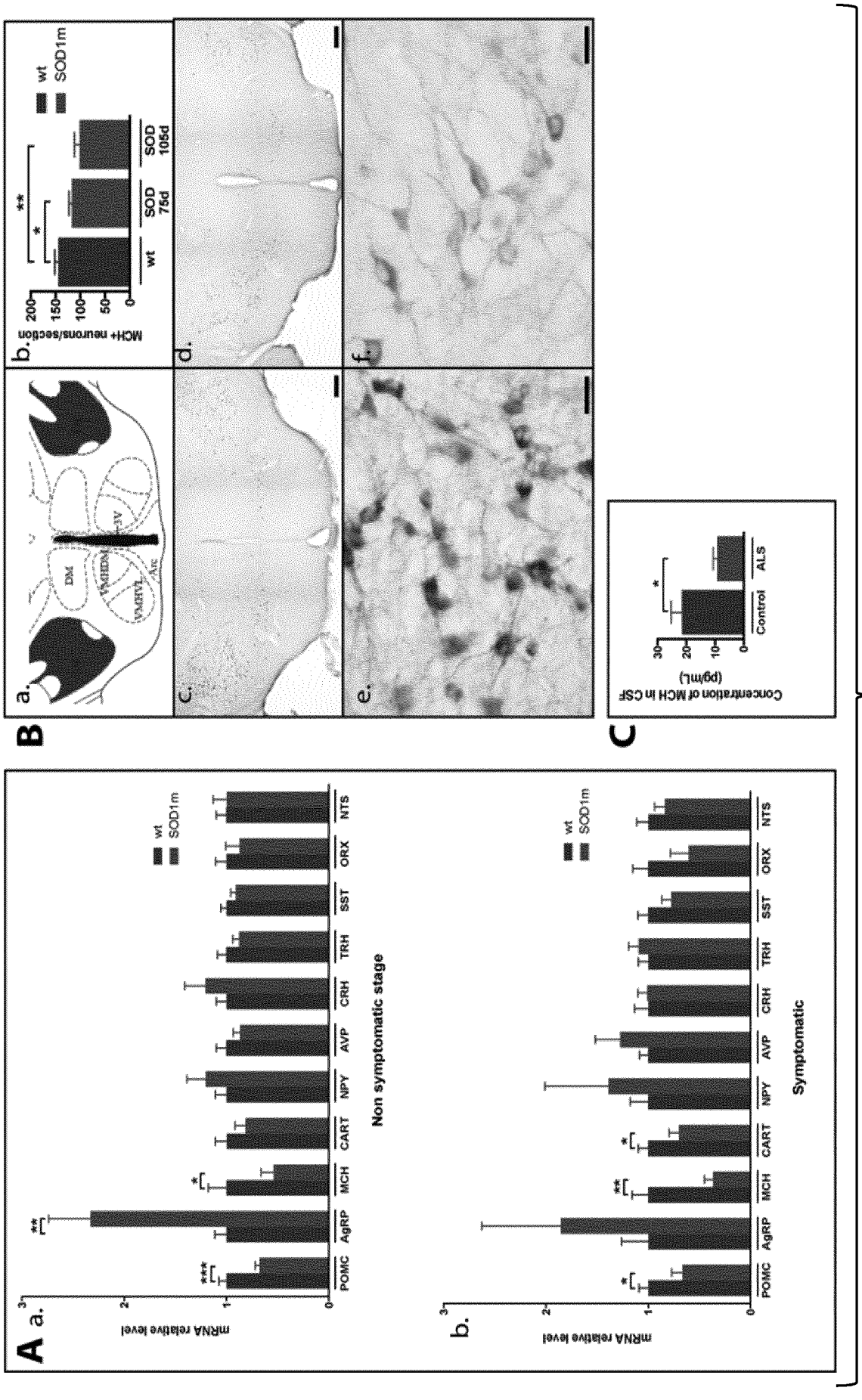

A
a.
mRNA relative level
wt
SOD1m
POMC
AgRP
MCH
CART
NPY
AVP
CRH
TRH
SST
ORX
NTS
Non symptomatic stage
b.
Symptomatic
B
a.
DM
VMHDM
VMHVL
Arc
3V
b.
MCH+ neurons/section
wt
SOD 75d
SOD 105d
c.
d.
e.
f.
C
Concentration of MCH in CSF (pg/mL)
Control
ALS

FIG.4

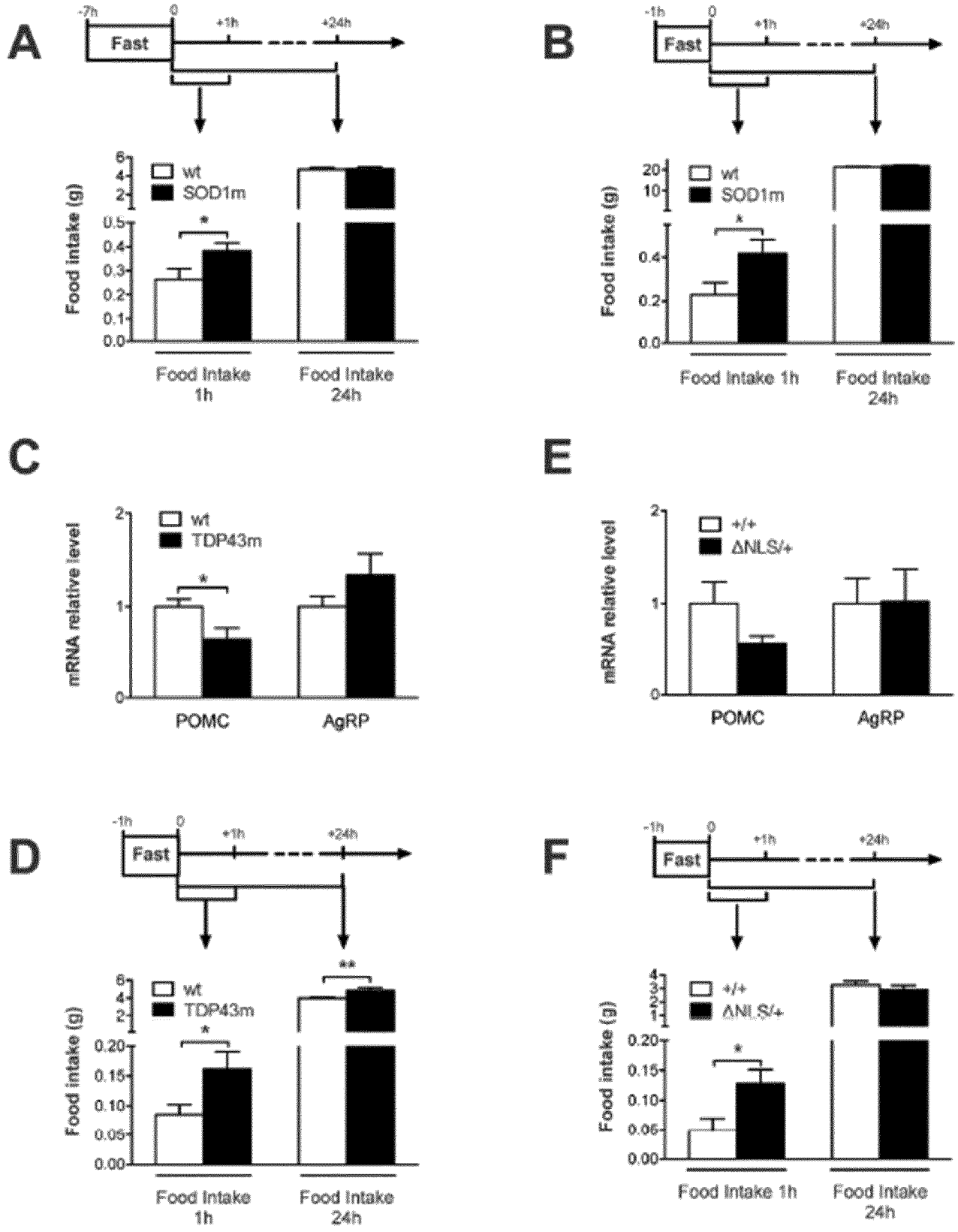

A
-7h
0
+1h
+24h
Fast
wt
SOD1m
Food intake (g)
Food Intake 1h
Food Intake 24h
B
-1h
0
+1h
+24h
Fast
wt
SOD1m
Food intake (g)
Food Intake 1h
Food Intake 24h
C
wt
TDP43m
mRNA relative level
POMC
AgRP
E
+/+
ΔNLS/+
mRNA relative level
POMC
AgRP
D
-1h
0
+1h
+24h
Fast
wt
TDP43m
Food intake (g)
Food Intake 1h
Food Intake 24h
F
-1h
0
+1h
+24h
Fast
+/+
ΔNLS/+
Food intake (g)
Food Intake 1h
Food Intake 24h

FIG.5

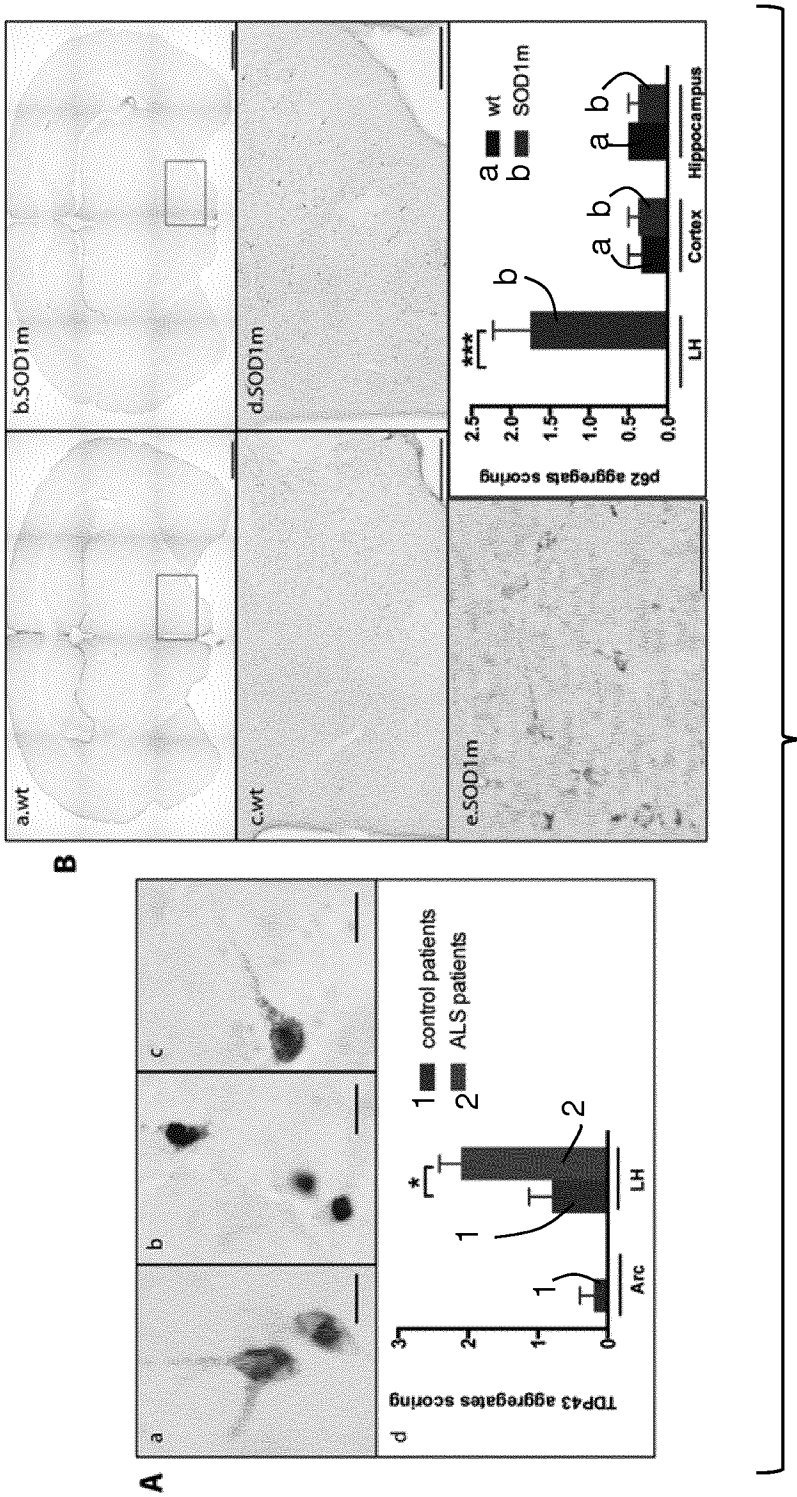

A
a
b
c
d
TDP43 aggregates scoring
control patients
ALS patients
Arc
LH
B
a.wt
b.SOD1m
c.wt
d.SOD1m
e.SOD1m
p62 aggregats scoring
wt
SOD1m
LH
Cortex
Hippocampus

FIG.6

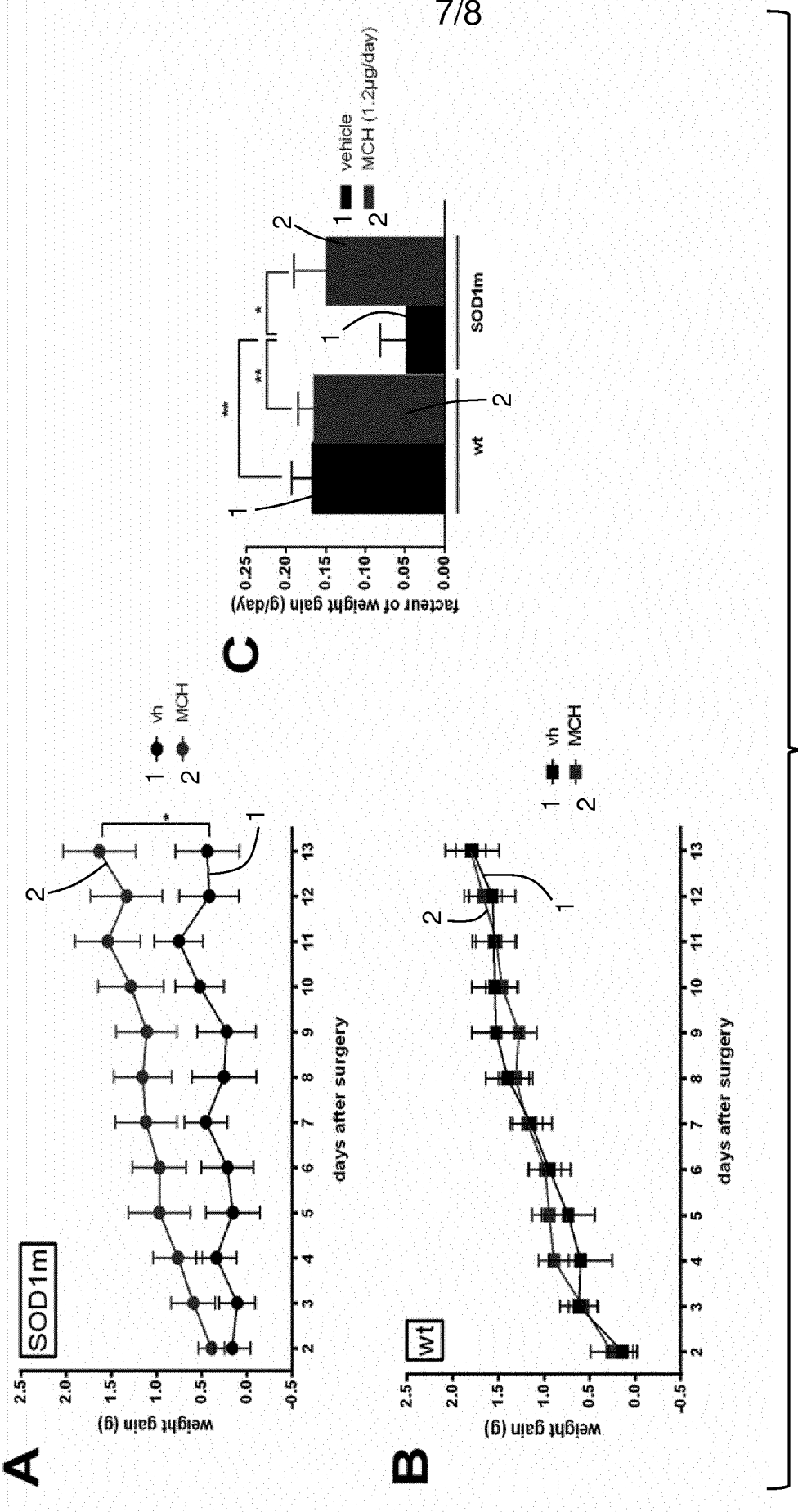

A
SOD1m
weight gain (g)
days after surgery
vh
MCH
B
wt
weight gain (g)
days after surgery
vh
MCH
C
factor of weight gain (g/day)
vehicle
MCH (1.2µg/day)
wt
SOD1m

FIG.7

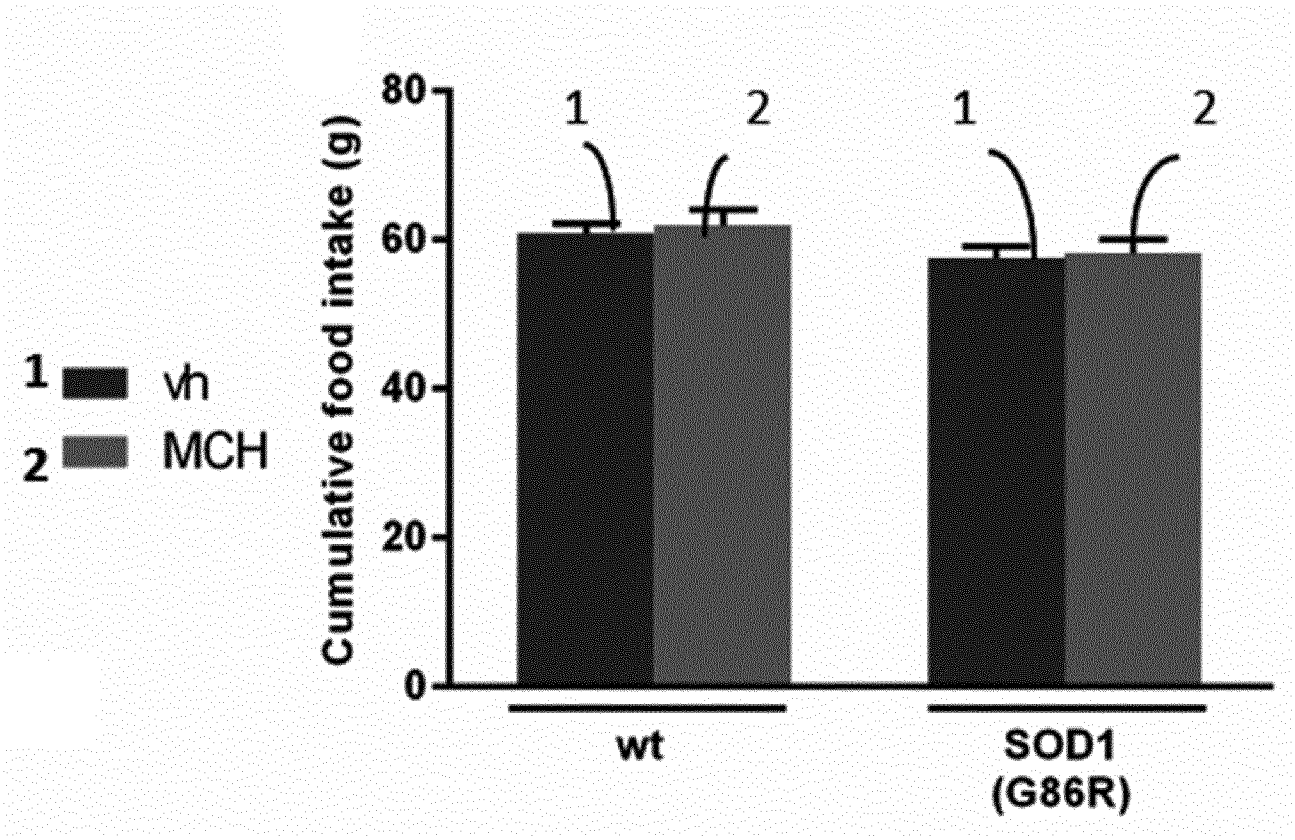

1 vh
2 MCH
Cumulative food intake (g)
80
60
40
20
0
1
2
1
2
wt
SOD1
(G86R)

FIG.8

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/081602

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/34 A61P25/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96/39162 A1 (JOSLIN DIABETES CENTER INC [US]; MARATOS FLIER ELEFTHERIA [US]) 12 December 1996 (1996-12-12) claims 1-5	1-9, 11-15
A	MASAKO SHIMADA ET AL: "Mice lacking melanin-concentrating hormone are hypophagic and lean", NATURE, NATURE PUBLISHING GROUP, UNITED KINGDOM, vol. 396, 17 December 1998 (1998-12-17), pages 670-674, XP002308086, ISSN: 0028-0836, DOI: 10.1038/25341 the whole document	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 March 2017	15/03/2017

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Rodrigo-Simón, Ana

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROSSI M ET AL: "Melanin-concentrating hormone acutely stimulates feeding, but chronic administration has no effect on body weight", ENDOCRINOLOGY, THE ENDOCRINE SOCIETY, US, vol. 138, no. 1, 1 January 1997 (1997-01-01), pages 351-355, XP002535522, ISSN: 0013-7227, DOI: 10.1210/EN.138.1.351 page 353, column 2, paragraph 3 the whole document	1-9, 11-15
Y	PRESSE F ET AL: "Melanin-concentrating hormone is a potent anorectic peptide regulated by food-deprivation and glucopenia in the rat", NEUROSCIENCE, NEW YORK, NY, US, vol. 71, no. 3, 1 January 1996 (1996-01-01), pages 735-745, XP002535523, ISSN: 0306-4522, DOI: 10.1016/0306-4522(95)00481-5 the whole document	1-9, 11-15
Y	DATABASE BIOSIS, [Online] 1 January 1996 (1996-01-01), ROVERE CAROLE ET AL: "Impaired processing of brain proneurotensin and promelanin-concentrating hormone in obese fat/fat mice", XP002535524, retrieved from BIOSIS Database accession no. PREV199699091708 the whole document	1-9, 11-15
Y	GONZALEZ DE AGUILAR J L ET AL: "The metabolic hypothesis in amyotrophic lateral sclerosis: insights from mutant Cu/Zn-superoxide dismutase mice", BIOMEDICINE AND PHARMACOTHERAPY, ELSEVIER, FR, vol. 59, no. 4, 1 May 2005 (2005-05-01), pages 190-196, XP027640142, ISSN: 0753-3322 [retrieved on 2005-05-01]	1-9, 11-15
A	the whole document	10

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DUPUIS L ET AL: "Evidence for defective energy homeostasis in amyotrophic lateral sclerosis: Benefit of a high-energy diet in a transgenic mouse model", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 101, no. 30, 27 July 2004 (2004-07-27), pages 11159-11164, XP002436994, ISSN: 0027-8424, DOI: 10.1073/PNAS.0402026101	1-9, 11-15
A	abstract	10

Y	WO 2010/148409 A1 (KNOPP NEUROSCIENCES INC [US]) 23 December 2010 (2010-12-23)	1-9, 11-15
A	the whole document	10

Y	US 2005/019423 A1 (KANE PATRICIA [US] ET AL) 27 January 2005 (2005-01-27)	1-9, 11-15
A	the whole document	10

Y	Robert G. Miller et al: "Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND)", Cochrane Database of Systematic Reviews , 14 March 2012 (2012-03-14), XP002756542, Retrieved from the Internet: URL:http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD001447.pub3/abstract?systemMessage=Subscribe+and+renew+is+currently+unavailable+online.+Please+contact+customer+care+to+place+an+order%3A++http%3A%2F%2Folabout.wiley.com%2FWileyCDA%2FSection%2Fid-397203.html++.Apologies+for+the+inconvenience. [retrieved on 2016-04-14]	1-9, 11-15
A	abstract	10

A	Marin et al.: "Alteration of nutritional status at diagnosis is a prognostic factor for survival of amyotrophic lateral sclerosis patients.", Journal of neurology, neurosurgery and psychiatry , 2011, XP002756541, Retrieved from the Internet: URL:http://www.ncbi.nlm.nih.gov/pubmed/21097551 [retrieved on 2016-04-13] the whole document	1-15

	-/--	

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CARLESI C1 ET AL: "Strategies for clinical approach to neurodegeneration in Amyotrophic lateral sclerosis", ARCHIVES ITALIENNES DE BIOLOGIE,, vol. 149, 1 January 2011 (2011-01-01), pages 151-167, XP009189514,	1-9, 11-15
A	the whole document	10

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/081602

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		CO 6480953 A2	16-07-2012
		EP 2442655 A1	25-04-2012
		JP 2012530723 A	06-12-2012
		RU 2012101792 A	27-07-2013
		US 2011009460 A1	13-01-2011
		US 2013245081 A1	19-09-2013
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