



(51) International Patent Classification:

A01K 67/027 (2006.01) A61K 49/00 (2006.01)
C12N 9/02 (2006.01)

(21) International Application Number:

PCT/EP2015/073909

(22) International Filing Date:

15 October 2015 (15.10.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

14189038.4 15 October 2014 (15.10.2014) EP

(71) Applicants: **FUNDACIÓ HOSPITAL UNIVERSITARI VALL D'HEBRON - INSTITUT DE RECERCA** [ES/ES]; Passeig Vall d'Hebron, 119-129, E-08035 Barcelona (ES). **INSTITUCIÓ CATALANA DE RECERCA I ESTUDIS AVANÇATS** [ES/ES]; Pg. Lluís Companys, 23, 3^a pta., E-08010 Barcelona (ES). **CENTRO DE INVESTIGACIÓN BIOMÉDICA EN RED ENFERMEDADES NEURODEGENERATIVAS (CIBERNED)** [ES/ES]; C/ Valderrebollo núm. 5, E-28031 Madrid (ES).

(72) Inventors: **VILA BOVER, Miquel**; C/ Muntaner, 187, pral. 2^a, E-08036 Barcelona (ES). **CARBALLO CARBAL, Iria**; C/ Girona, 150, 1^o 4^a, E-08037 Barcelona (ES). **BOVÉ BADELL, Jordi**; C/ Balmes, 391, 3^o 1^a, E-08022 Barcelona (ES). **CUADROS ARASA, Thais**; C/ Camp del ferro, 1-3, 1^o 11, E-08027 Barcelona (ES). **LAGUNA TUTSET, Ariadna**; Travessera de Gràcia 185, 2^a 3^a, E-08012 Barcelona (ES).

(74) Agent: **ZBM PATENTS - ZEA, BARLOCCI & MARKVARDSEN**; Pl. Catalunya, 1 2nd floor, 08002 Barcelona (ES).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))

Published:

— with international search report (Art. 21(3))
— with sequence listing part of description (Rule 5.2(a))

(54) Title: HUMANIZED NEUROMELANIN-CONTAINING RODENT

(57) Abstract: The invention provides a humanized rodent exhibiting age-dependent production and intracellular accumulation of human-like neuromelanin, up to levels reached in elder humans, within Parkinson's disease-vulnerable neuronal groups. The humanized neuromelanin-containing rodent stably expresses the human tyrosinase gene in at least the dopaminergic neurons of the substantia nigra pars compacta and can be a wild-type rodent to which the human tyrosinase gene has been transduced via a viral vector, or a transgenic rodent that expresses the human gene in a constitutive or inducible form. In any of these cases, the rodent develops features of Parkinson's disease such as nigro-striatal dopaminergic neurodegeneration and motor impairment, making it a valuable tool for the (i) search of biomarkers for the early detection, diagnosis or progression of the disease, and (ii) the screening of new therapies with disease-modifying potential to combat PD.



WO 2016/059166 A1

Humanized neuromelanin-containing rodent

The present invention provides a rodent that expresses the human tyrosinase gene in the brain, an enzyme which might be involved in the production of neuromelanin, and accumulates the latter pigment in dopaminergic neurons. This rodent has potential applications in the pre-clinical research of Parkinson's disease (PD), enabling: (i) the search of potential neuromelanin-related biomarkers for early detection, diagnosis and/or progression of PD, and (ii) the screening for treatments aimed at modulating neuromelanin accumulation to eventually slow down or halt the progression of PD.

BACKGROUND ART

Parkinson's disease is one of the main degenerative diseases of the central nervous system, second only to Alzheimer's. It is estimated to affect around 7 million people worldwide, and its prevalence is estimated to be around 0.3% in the industrialized nations. It is characterized by a progressive and sustained neurodegeneration in different brain regions, mostly of dopaminergic neurons in the substantia nigra pars compacta (SNPC), located in the midbrain. This neuronal death causes a variety of symptoms which become more severe with time, such as motor impairment, dementia and depression. PD is treated with Levodopa, dopamine agonists, anticholinergics and MAO inhibitors, among others. However, there is currently no cure for the disease. Therefore, new disease-modifying treatments are badly needed.

The exact mechanism by which the dopamine-producing neurons die in PD is not known. However it is widely accepted that there exists a selective vulnerability of the groups of neurons that contain the pigment neuromelanin (NM), which are basically those of the SNPC, and to a lesser extent the noradrenergic neurons of the locus coeruleus (LC) and dorsal motor nucleus of the vagus (DMNV). The mechanisms of synthesis, accumulation pattern and function of NM are currently a matter of debate, as there are conflicting disclosures on whether NM is a by-product resulting from catecholamines auto-oxidation or whether its synthesis is enzymatically regulated (Ikemoto, K. et.al. "Does tyrosinase exist in neuromelanin-pigmented neurons in the human substantia nigra?" *Neurosc. Lett.* 1998, vol. 11, pp. 198-200; Tief, K., et.al. "Tyrosinase, the key enzyme in melanin synthesis, is expressed in murine

brain" Eur. J. Biochem. 1996, vol. 241, pp. 12-16; Xu, Y., et.al. "Tyrosinase mRNA is expressed in human substantia nigra" Brain Res. Mol. Brain Res. 1997, vol. 45, pp.159-162; Greggio E., et. al. "Tyrosinase exacerbates dopamine toxicity but is not genetically associated with Parkinson's disease" J. of Neurochem. 2005, vol. 93, pp. 246-256). Furthermore, dying neurons in PD are also characterized by the presence of cytoplasmic protein inclusions known as Lewy bodies (LB), whose main component is the protein α -synuclein. The latter has been extensively studied, and its precipitation, misfolding and aggregation is now considered to be one of the main culprits in the development of at least some forms of PD (Olanow, W., et. al. "Parkinson's disease and alpha synuclein: Is Parkinson's disease a prion-like disorder?" Movement Disorders 2013, vol. 28, pp. 31-40). Furthermore, NM has been shown to associate to lipid content, oxidation or iron loading, reactions known to precipitate α -synuclein. In fact, several studies have reported that α -synuclein accumulation occurs around the NM granules (Fasano, M, et al. "Residual substantia nigra neuromelanin in Parkinson's disease is cross-linked to α -synuclein". Neurochem Int 2003, vol. 42, pp. 603–606); Halliday, G. M. et al. " α -Synuclein redistributes to neuromelanin lipid in the substantia nigra early in Parkinson's disease". Brain 2005, vol. 128, pp. 2654-2664).

Aging is one of the main risk factors associated to the development of PD. In humans, NM progressively accumulates with age since it cannot be degraded or eliminated by neurons. However, the potential contribution of the accumulation of NM to PD is not known mainly due to the fact that, unlike humans, common experimental animal species used in pre-clinical studies (mainly rodents) lack NM. In fact, the great abundance of NM in the SNPC seems to be characteristic of humans, as a macroscopic dark pigmentation of the midbrain is not observed in other species or NM is only present at moderate levels in some species of monkeys, horses or sheep.

Many animal models used for studying PD are based on the exposure to neurotoxic compounds such as 6-OHDA and MPTP (Blum, D., et.al. "Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease" Prog. In Neurobiol. 2001, vol. 65, pp. 135-172) whose effects mimic, at least partially, clinical or neuropathological aspects of the disease. Several transgenic rodent

models targeting PD-causative genes have also been described, including transgenics for DJ-1, LRRK2, Parkin, PINK1 and α -synuclein (Harvey, B.K. et. al. "Transgenic rodent models of Parkinson's disease" 2008, vol. 101, pp. 89-92), as well as for other PD-related non-causative genes. All these models have enormously contributed to getting a deeper insight into the familial as well as the sporadic forms of the disease.

In spite of these efforts, so far there is no animal model for studying the potential contribution of NM accumulation in the development and progression of PD. Therefore, a factor so intimately linked to PD such as NM is not taken into account in current experimental paradigms of PD. Thus, there is a long felt need to develop rodent models which recapitulate NM synthesis and accumulation over time within catecholaminergic PD-vulnerable neuronal regions, as it occurs in humans. Besides throwing light into the potential role of NM in PD, this model would serve as a useful platform for (i) the search of potential neuromelanin-related biomarkers for the early detection, diagnosis and/or progression of PD, and (ii) the screening of novel PD therapies aimed at modulating neuromelanin accumulation.

SUMMARY OF THE INVENTION

Inventors have surprisingly found that a rodent that expresses the human tyrosinase gene in the brain, and more specifically in the SNPC, not only accumulates NM in its neurons, but also reveals physiological changes at the cellular level that correspond to those seen in the neurons of PD patients, and what is even more remarkable, it additionally displays motor dysfunctions and neurodegeneration typical of PD.

This is a striking result for several reasons. On the one hand, as stated above, there is a high degree of controversy surrounding the way NM is synthesized/produced in the neuron. On the other hand, the accumulation of NM is not an established cause of neuronal dysfunction. Finally, the accumulation of a pigment such as NM in the neurons of a rodent would, *a priori*, have unpredictable consequences in view of what is known in the field.

It has been remarkably found that the rodent of the invention does accumulate NM progressively with age as is the case of human subjects, that the

accumulation takes place in the dopaminergic neurons (especially those of the SNPC) and further, that it is significant enough to surpass a certain pathologic threshold as it ultimately is linked with the appearance of neurodegeneration and motor dysfunction typically experienced by PD patients.

5

The rodent that is part of the invention can thus be used as a faithful pre-clinical model of PD, and be exploited as a screening tool for the search of potential neuromelanin-related biomarkers for the early detection, diagnosis and/or progression of PD, and for the testing of new therapies with disease-modifying potential that combat NM accumulation as a potential mechanism of action with which to treat PD.

10

Thus, a first aspect of the invention is a rodent that comprises the human tyrosinase gene, the gene being expressed in at least the dopaminergic neurons of the substantia nigra pars compacta.

15

A second aspect of the invention is a method of screening for therapeutic agents that prevent, delay or halt the development of neurodegenerative disease in the rodent of the first aspect of the invention, comprising: 1) exposing said rodent to at least one candidate therapeutic agent; and 2) assessing the effect of the at least one therapeutic agent on the progression of neurodegenerative disease in said rodent.

20

A third aspect of the invention is a method of screening for a biomarker for the early detection, diagnosis and/or prognosis of neurodegenerative disease in the rodent of the first aspect of the invention, comprising: 1) isolating from said rodent and a second reference rodent at least one sample; 2) determining the differential expression and/or level of a potential biomarker between said rodent and the second reference rodent, wherein if there is a difference in expression and/or level of the potential biomarker, then the potential biomarker is taken as a biomarker for the early detection, diagnosis and/or prognosis of neurodegenerative disease.

25

30

It is also part of the invention a nucleotide sequence or gene construct comprising the human tyrosinase gene under the control of or operably linked to a promoter of a tyrosine hydroxylase gene. The invention also encompasses vectors comprising the above mentioned nucleotide sequence,

35

as well as host cells in the rodent comprising said vectors or said nucleotide sequence

It is also a part of the invention a set of separate/independent nucleotide sequences, comprising: (a) a sequence that comprises the human tyrosinase gene operably linked to an inducible promoter, and (b) a nucleotide sequence comprising a neuron specific expression promoter (in particular a rodent tyrosine hydroxylase promoter) linked to a sequence the expression of which promotes expression of the adjacent gene, in this case human tyrosinase (a) by interaction with the inducible promoter.

All these gene constructs comprise sequences with the human tyrosinase gene sequence and also sequences promoting the expression of the human tyrosinase gene in neurons. By promoting it is to be understood that the expression is neuron specific or only achieved in some brain tissue types.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG.1. Human-like NM production in the SNpc of AAV-hTyr-injected rats. (a) *Left panel*, adult Sprague-Dawley rats received a single unilateral stereotaxic injection with 2 μ l of AAV2/1-CMV-hTyr into the region immediately above the right SNpc. *Right panel*, by 4 weeks post-injection, 80% of ipsilateral DA SNpc neurons (*TH*, *top right*) were transduced with the tyrosinase gene (*Tyr*, *middle right*). The image in the bottom right panel is a composite of the top and middle images. (b) Similar to humans, accumulation of NM brown pigment was observed macroscopically in unstained ipsilateral SNpc of AAV-hTyr-injected rats at 4 weeks post-injection (darkened area, dashed line). No NM accumulation was observed in contralateral (control) hemisphere (identified by a puncture), as rodents normally lack this brain pigment. (c) Ipsilateral SNpc pigmentation in AAV-hTyr-injected rats corresponds to the presence of intracellular NM within SNpc/VTA neurons, as shown microscopically in a 30 μ m-thick unstained section from the same brain. Whereas tyrosinase was widely expressed throughout the injected hemisphere, accumulation of NM was restricted to the SNpc and VTA, which mimics the pattern of NM distribution in humans. (d) By 8 weeks post-AAV-hTyr injection, ipsilateral SNpc neurons from injected rats exhibited intracellular NM levels similar to that of elder human brains, with NM occupying most of their cellular cytoplasm

(Hematoxylin&Eosin staining). (e) As in humans, pigmented SNpc from AAV-hTyr-injected rodents can be detected (arrow) by NM-sensitive high-resolution T1-weighted magnetic resonance imaging (MRI; data obtained *ex-vivo* from a mouse brain at 16 weeks post-AAV-hTyr injection). (f) Electron micrographs of NM from human LC of a 81-year-old subject (*top*) and an AAV-hTyr-injected rat SNpc at 16 weeks post-injection (*bottom*), both exhibiting strikingly similar NM-containing autophagic-related vacuoles displaying NM (electron dense matrix) and characteristic lipid droplets (asterisks). Scale bar, 1 μ m (human), 500 nm (rat).

FIG.2. PD-like neuropathological features in AAV-hTyr-injected rats. By 16 weeks post-AAV-hTyr injection, ipsilateral rat SNpc (*bottom panels*) exhibit several neuropathological features typical of PD brains (*top panels*), including extracellular (E NM) and perivascular NM (P NM), neuronophagia (NP), Marinesco bodies (MB, arrows) and pale bodies (PB, LB precursors, arrowheads). Scale bars, 10 μ m (except *b*, 20 μ m).

FIG.3. SNpc dopaminergic neuronal dysfunction/degeneration in AAV-hTyr-injected rats. (*Top panel – Forepaw akinesia*). The x-axis represents weeks after the AAV-hTyr injection and the y-axis represents the Contralateral forepaw contacts – CFC (% of total contacts). Progressive reduction of contralateral forelimb use in AAV-Tyr-injected rats, starting at 4 weeks post-AAV-hTyr injection and reaching statistical significance by 8 weeks and onwards, as assessed with the cylinder test. Animals were also tested prior to AAV-Tyr injections (basal) to exclude putative spontaneous asymmetry. (*Bottom panel – Number of SNpc Tyrosine Hydroxylase (TH)-positive neurons*) The x-axis represents weeks after the AAV-hTyr injection and the y-axis represents the number of SNpc TH-positive neurons (ipsilateral to the injected side) – SNpc TH+ . Stereological quantification of the number of TH-positive neurons in the SNpc of rats injected with the AAV-Tyr at different times post-injection (from 2 weeks to 2 years), indicating a progressive loss of TH-positive neurons. *N*=6-8 animals per group. **p*<0.05, compared to control animals (basal); #*p*<0.05, compared to 8 weeks and 12 months post-AAV-hTyr injection (one way ANOVA, Student-Newmann-Keuls post-hoc test).

FIG.4. NM accumulation in catecholaminergic brain structures of 12 month-old Tg-TH-hTyr mice. (*Left and middle panels*) Nissl-stained cryosections showing

intracellular accumulation of NM (*dark brown*) in the cytoplasm of neurons from the substantia nigra pars compacta (SNpc), ventral tegmental area (VTA) and locus coeruleus (LC) regions. (*Right panel*) Digoxigenin-based in situ hybridization for human *tyrosinase* showing expression of hTyr in SNpc, VTA and LC neurons of Tg-TH-hTyr mice.

DETAILED DESCRIPTION OF THE INVENTION

The human tyrosinase gene is a copper-containing enzyme that catalyzes the production of melanin and other pigments from tyrosine oxydation. In humans, it is encoded by the *TYR* gene. Its protein product is found in the Uniprot database with the entry P14679 (last update on 11.06.2014). The sequence of the human tyrosinase gene used in the present invention is (SEQ ID NO 1):

```

10  GACCTTGTGAGGACTAGAGGAAGAATGCTCCTGGCTGTTTTGTACTGCCTGCTGTGG
    AGTTTCCAGACCTCCGCTGGCCATTTCCCTAGAGCCTGTGTCTCCTCTAAGAACCTG
15  ATGGAGAAGGAATGCTGTCCACCGTGGAGCGGGACAGGAGTCCCTGTGGCCAGCTT
    TCAGGCAGAGGTTCCCTGTCAGAATATCCTTCTGTCCAATGCACCACTTGGGCCTCAA
    TTTCCCTTCACAGGGGTGGATGACCGGGAGTCGTGGCCTTCCGTCTTTTATAATAGG
    ACCTGCCAGTGCTCTGGCAACTTCATGGGATTCAACTGTGGAACTGCAAGTTTGGC
20  TTTTGGGGACCAAACCTGCACAGAGAGACGACTCTTGGTGAGAAGAAACATCTTCGAT
    TTGAGTGCCCCAGAGAAGGACAAATTTTTTGCCTACCTCACTTTAGCAAAGCATACC
    ATCAGCTCAGACTATGTCATCCCCATAGGGACCTATGGCCAAATGAAAAATGGATCA
    ACACCCATGTTTAACGACATCAATATTTATGACCTCTTTGTCTGGATGCATTATTAT
    GTGTCAATGGATGCACTGCTTGGGGGATCTGAAATCTGGAGAGACATTGATTTTGCC
25  CATGAAGCACCAGCTTTTCTGCCTTGGCATAGACTCTTCTTGTTGCGGTGGGAACAA
    GAAATCCAGAAGCTGACAGGAGATGAAAACCTCACTATTCCATATTGGGACTGGCGG
    GATGCAGAAAAGTGTGACATTTGCACAGATGAGTACATGGGAGGTCAGCACCCACACA
    AATCCTAACTTACTCAGCCCAGCATCATTCTTCTCCTCTTGGCAGATTGTCTGTAGC
    CGATTGGAGGAGTACAACAGCCATCAGTCTTTATGCAATGGAACGCCCAGGGGACCT
30  TTACGGCGTAATCCTGGAAACCATGACAAATCCAGAACCCCAAGGCTCCCCTCTTCA
    GCTGATGTAGAATTTTGCCTGAGTTTGACCCAATATGAATCTGGTTCCATGGATAAA
    GCTGCCAATTTTCACTTTAGAAATACACTGGAAGGATTTGCTAGTCCACTTACTGGG
    ATAGCGGATGCCTCTCAAAGCAGCATGCACAATGCCTTGCACATCTATATGAATGGA
    ACAATGTCCCAGGTACAGGGATCTGCCAACGATCCTATCTTCTTCTTCACCATGCA
35  TTTGTTGACAGTATTTTTGAGCAGTGGCTCCGAAGGCACCGTCCTCTTCAAGAAGTT
    TATCCAGAAGCCAATGCACCCATTGGACATAACCGGGAATCCTACATGGTTCCTTTT
    ATACCACTGTACAGAAATGGTGATTTCTTTATTTTCATCCAAAGATCTGGGCTATGAC

```

TATAGCTATCTACAAGATTCAGACCCAGACTCTTTTCAAGACTACATTAAGTCCTAT
 TTGGAACAAGCGAGTCGGATCTGGTCATGGCTCCTTGGGGCGGCGATGGTAGGGGCC
 GTCCTCACTGCCCTGCTGGCAGGGCTTGTGAGCTTGCTGTGTTCGTCACAAGAGAAAG
 CAGCTTCCTGAAGAAAAGCAGCCACTCCTCATGGAGAAAGAGGATTACCACAGCTTG
 5 TATCAGAGCCATTTATAAAAGGCTTAGGCAATAGAGTAGGGCCAAAAAGCCTGACCT
 CACTCTAACTCAAAGTAATGTCCAGGTTCCAGAGAATATCTGCTGGTATTTTTCTG
 TAAAGACCATTTGCAAAATTGTAACCTAATACAAAGTGTAGCCTTCTTCCAACCTCAG
 GTAGAACACACCTGTCTTTGTCTTGCTGTTTTCACTCAGCCCTTTTAACATTTTCCC
 CTAAGCCC

10

For the sake of understanding, the following definitions are included.

The term “expresses” when referring to a gene, is to be understood here as the process by which the information stored in the DNA of the gene is used for the synthesis of a functional gene product (usually a protein, but can also sometimes be an RNA). In the case of the human tyrosinase, the expression of the human tyrosinase gene is to be understood as the production of the human tyrosinase enzyme.

20 The term “progressively accumulates” as used herein refers to the accumulation of NM along the lifetime of the rodent animal in such a way that the rodent of young age features a certain amount of NM in its dopaminergic neurons, the adults feature higher amounts than the young rodents, and the older animals are the ones featuring the highest amounts of NM, mimicking the progression of NM accumulation in humans, that is, the older the animal is, the higher the amount of intracellular NM.

The term “transduction” as used herein refers to the process whereby heterologous (foreign) DNA is introduced into a host cell via a viral vector. The aim of this process is for the piece of DNA to be integrated in the genome of the host cell so that the protein or RNA that the foreign DNA codes for is stably expressed in the host cell. In the case of the present invention, the transduction of the human tyrosinase gene is carried out with the aim of having the host cell (and especially the neurons of the SNPC of a rodent) stably expressing the h-tyrosinase gene.

35

The term “viral vector” as used herein refers to a virus that is used as a

delivery device to insert heterologous genetic material into a host cell (transduction).

The term “adeno-associated virus (AVV)” as used herein refers to a viral vector that infects both dividing and quiescent primate (and human) cells. Because they seem to lack any pathogenic effects, and usually integrate in the same place of the genome (the AVVS1 site, in chromosome 19), this viral vectors can safely be used to transduce foreign DNA into human cells in gene therapy applications. The “AVV-Tyr” of the present invention is an adeno-associated viral vector that bears the human tyrosinase gene. In particular, the full sequence of the transduction vector used in the present invention (AVV2/1-CMV-TYR) is SEQ ID NO 2 (the coding sequence for the human tyrosinase gene is highlighted in bold):

```
CAGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTCGG
15 GCGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCC
AACTCCATCACTAGGGGTTTCCTTGTTAGTTAATGATTAACCCGCCATGCTACTTATCT
ACGTAGCCATGCTCTAGACATGGCTCGACAGATCTCAATATTGGCCATTAGCCATAT
TATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATACGTTGT
ATCTATATCATAATATGTACATTTATATTGGCTCATGTCCAATATGACCGCCATGTT
20 GGCATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC
CAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCCACT
TGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACG
25 GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTTGGCAGT
ACACCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCA
TTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTCCAAAATGTC
GTAACAACTGCGATCGCCCGCCCCGTTGACGCAAATGGGCGGTAGGCGTGACGGTG
30 GGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCACTAGAAGCTTTAT
TGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAGTGCTTCTGACACAACAGT
CTCGAACTTAAGCTGCAGTGACTCTCTTAAGGTAGCCTTGACAGAGTTGGTCGTGAG
GCACTGGGCAGGTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAAC
TGGGCTTGTCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTTA
35 CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCAATTACAGCT
CTTAAGGCTAGAGTACTTAATACGACTCACTATAGGCTAGCctcgacctcgagacgc
gtgataaacttaagcttggtaccgagctcggatccactagtcagtggtggaatt
```

ctgcagatatccagcacagtggcgccgctcgacc**GACCTTGTGAGGACTAGAGGAA**
GAATGCTCCTGGCTGTTTTGTACTGCCTGCTGTGGAGTTTCCAGACCTCCGCTGGCC
ATTTCCCTAGAGCCTGTGTCTCCTCTAAGAACCTGATGGAGAAGGAATGCTGTCCAC
CGTGGAGCGGGGACAGGAGTCCCTGTGGCCAGCTTTCAGGCAGAGGTTCTGTGAGA
5 **ATATCCTTCTGTCCAATGCACCACCTTGGGCCTCAATTTCCCTTCACAGGGGTGGATG**
ACCGGGAGTCGTGGCCTTCCGTCTTTTATAATAGGACCTGCCAGTGCTCTGGCAACT
TCATGGGATTCAACTGTGGAACTGCAAGTTTGGCTTTTGGGGACCAAACCTGCACAG
AGAGACGACTCTTGGTGAGAAGAAACATCTTCGATTTGAGTGCCCCAGAGAAGGACA
AATTTTTTGCCTACCTCACTTTAGCAAAGCATAACCATCAGCTCAGACTATGTCATCC
10 **CCATAGGGACCTATGGCCAAATGAAAAATGGATCAACACCCATGTTTAACGACATCA**
ATATTTATGACCTCTTTGTCTGGATGCATTATTATGTGTCAATGGATGCACTGCTTG
GGGGATCTGAAATCTGGAGAGACATTGATTTTGCCCATGAAGCACCAGCTTTTCTGC
CTTGGCATAGACTCTTCTTGTGCGGTGGGAACAAGAAATCCAGAAGCTGACAGGAG
ATGAAAACCTTCACTATTCCATATTGGGACTGGCGGGATGCAGAAAAGTGTGACATTT
15 **GCACAGATGAGTACATGGGAGGTGAGCACCCCAAAATCCTAACTTACTCAGCCCAG**
CATCATTTCTTCTCCTCTTGGCAGATTGTCTGTAGCCGATTGGAGGAGTACAACAGCC
ATCAGTCTTTATGCAATGGAACGCCCGAGGGACCTTTACGGCGTAATCCTGGAAACC
ATGACAAATCCAGAACCCCAAGGCTCCCCTCTTCAGCTGATGTAGAATTTTGCCTGA
GTTTGACCCAATATGAATCTGGTTCCATGGATAAAGCTGCCAATTTAGCTTTAGAA
20 **ATACACTGGAAGGATTTGCTAGTCCACTTACTGGGATAGCGGATGCCTCTCAAAGCA**
GCATGCACAATGCCTTGACATCTATATGAATGGAACAATGTCCCAGGTACAGGGAT
CTGCCAACGATCCTATCTTCTTCTTCAACCATGCATTTGTTGACAGTATTTTTGAGC
AGTGGCTCCGAAGGCACCGTCTTCAAGAAGTTTATCCAGAAGCCAATGCACCCA
TTGGACATAACCGGGAATCCTACATGGTTCTTTTATACCACTGTACAGAAATGGTG
25 **ATTTCTTTATTTTATCCAAAGATCTGGGCTATGACTATAGCTATCTACAAGATTGAG**
ACCCAGACTCTTTTCAAGACTACATTAAGTCCTATTTGGAACAAGCGAGTCGGATCT
GGTCATGGCTCCTTGGGGCGCGATGGTAGGGGCCGTCCTCACTGCCCTGCTGGCAG
GGCTTGTGAGCTTGCTGTGTGTCGTCACAAGAGAAAGCAGCTTCTGAAGAAAAGCAGC
CACTCCTCATGGAGAAAGAGGATTACCACAGCTTGTATCAGAGCCATTTATAAAAGG
30 **CTTAGGCAATAGAGTAGGGCCAAAAGCCTGACCTCACTCTAACTCAAAGTAATGTC**
CAGGTTCCCAGAGAATATCTGCTGGTATTTTTCTGTAAAGACCATTTGCAAAATTGT
AACCTAATACAAAGTGTAGCCTTCTTCCAACCTCAGGTAGAACACACCTGTCTTTGTC
TTGCTGTTTTTCACTCAGCCCTTTTAAACATTTTCCCCTAAGCCCctagagggcccggtt
tateggatcccgggccggcgccgcTTCCCTTTAGTGAGGGTTAATGCTTCGAGCAGA
35 CATGATAAGATACATTGATGAGTTTGGACAAACCACAACCTAGAATGCAGTGAAAAAA
ATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTG
CAATAAACAAGTTAACAACAACAATTGCATTATTTTATGTTTCAGGTTTCAGGGGGA

GATGTGGGAGGTTTTTTAAAGCAAGTAAACCTCTACAAATGTGGTAAAATCCGATA
AGGGACTAGAGCATGGCTACGTAGATAAGTAGCATGGCGGGTTAATCATTA ACTACA
AGGAACCCCTAGTGATGGAGTTGGCCACTCCCTCTCTGCGCGCTCGCTCGCTCACTG
AGGCCGGGCGACCAAAGGTCGCCCAGCGCCGGGCTTTGCCC GGCGGCCTCAGTGA
5 GCGAGCGAGCGCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCC
CAACAGTTGCGCAGCCTGAATGGCGAATGGAATTCCAGACGATTGAGCGTCAAAATG
TAGGTATTTCCATGAGCGTTTTTCCGTTGCAATGGCTGGCGGTAATATTGTTCTGGA
TATTACCAGCAAGGCCGATAGTTTGAGTTCTTCTACTCAGGCAAGTGATGTTATTAC
TAATCAAAGAAGTATTGCGACAACGGTTAATTTGCGTGATGGACAGACTCTTTTACT
10 CGGTGGCCTCACTGATTATAAAAACACTTCTCAGGATTCTGGCGTACCGTTCCTGTC
TAAAATCCCTTTAATCGGCCTCCTGTTTAGCTCCCGCTCTGATTCTAACGAGGAAAG
CACGTTATACGTGCTCGTCAAAGCAACCATAGTACGCGCCCTGTAGCGGCGCATTA
GCGCGGCGGGTGTTGGTGGTTACGCGCAGCGTGACCGCTACACTTGCCAGCGCCCTAG
CGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTCTCGCCACGTTGCGCGGCTTTCCCC
15 GTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTTTACGGCACC
TCGACCCCAAAAACTTGATTAGGGTGATGGTTCACGTAGTGGGCCATCGCCCTGAT
AGACGGTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAGTGGACTCTTGT
TCCAAACTGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTGATTTATAAGGGA
TTTTGCCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAAATTTAACG
20 CGAATTTTAACAAAATATTAACGTCTACAATTTAAATATTTGCTTATACAATCTTCC
TGTTTTTTGGGGCTTTTCTGATTATCAACCGGGGTACATATGATTGACATGCTAGTTT
TACGATTACCGTTCATCGATTCTCTTGTTTGCTCCAGACTCTCAGGCAATGACCTGA
TAGCCTTTGTAGAGACCTCTCAAAAATAGCTACCCTCTCCGGCATGAATTTATCAGC
TAGAACGGTTGAATATCATATTGATGGTGATTTGACTGTCTCCGGCCTTTCTCACCC
25 GTTTGAATCTTTACCTACACATTACTCAGGCATTGCATTTAAAATATATGAGGGTTC
TAAAAATTTTTATCCTTGCGTTGAAATAAAGGCTTCTCCCGCAAAGTATTACAGGG
TCATAATGTTTTTGGTACAACCGATTTAGCTTTATGCTCTGAGGCTTTATTGCTTAA
TTTTGCTAATTCTTTGCCTTGCCTGTATGATTTATTGGATGTTGGAATCGCCTGATG
CGGTATTTTCTCCTTACGCATCTGTGCGGTATTTACACCGCATATGGTGCACTCTC
30 AGTACAATCTGCTCTGATGCCGCATAGTTAAGCCAGCCCCGACACCCGCCAACACCC
GCTGACGCGCCCTGACGGGCTTGTCTGCTCCCGGCATCCGCTTACAGACAAGCTGTG
ACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTACCGTCAATCACCGAAACGCGCG
AGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATG
GTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGT
35 TTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAA
ATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCC
CTTATTCCTTTTTTTGCGGCATTTTGCCTTCCTGTTTTTTGCTCACCCAGAAACGCTG

GTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTG
GATCTCAACAGCGGTAAGATCCTTGAGAGTTTTTCGCCCCGAAGAACGTTTTTCCAATG
ATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGACGCCGGG
CAAGAGCAACTCGGTGCGCCGATACACTATTCTCAGAATGACTTGGTTGAGTACTCA
5 CCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCT
GCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGA
CCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACCTCGCCTTGAT
CGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATG
CCTGTAGCAATGGCAACAACGTTGCGCAAACCTATTAACCTGGCGAACTACTTACTCTA
10 GCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTT
CTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAG
CGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATC
GTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATC
GCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACCTGTCAGACCAAGTTTACTCA
15 TATATACTTTAGATTGATTTAAACTTCATTTTTTAATTTAAAAGGATCTAGGTGAAG
ATCCTTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTTCGTTCCACTGA
GCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGC
GTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCG
GATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATA
20 CCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTA
GCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGC
GATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAG
CGGTGCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTAC
ACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGG
25 AGAAAGGCGGACAGGTATCCGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGG
GAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTC
TGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGAAAAAC
GCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATG
TTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGA
30 GCTGATACCGCTCGCCGACGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAA
GCGGAAGAGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAA
TG

The term “a gene is operably linked to the promoter” as used herein refers to a
35 functional linkage between two sequences, a first sequence which
corresponds to a promoter, and a second sequence, which in the invention at
hand can be the hTyr gene (the DNA coding for human tyrosinase) or the

gene coding for the tetracycline transactivator (tTA). The promoter sequence regulates transcription of the DNA corresponding to the second sequence. Generally, operably linked means that the two nucleic acid sequences being linked are contiguous and, when necessary, in the same reading frame.

5

The term "screening for therapeutic agents" as used herein refers to the use of the rodent that forms part of the invention for testing organic molecules, peptides, proteins, DNA, antisense oligonucleotide, small interfering RNA, therapeutic vaccines or any cell-based or radiation-based therapy for the treatment of any pathology related to the accumulation of NM, and especially PD.

10

The expression "screening for a biomarker" relates to the use of the rodent that forms part of the invention for determining whether a potential biomarker between said rodent and a second reference rodent, is differentially expressed and/or has different levels. If this is the case, then the potential biomarker is taken as a biomarker for the early detection, diagnosis and/or prognosis of neurodegenerative disease.

15

The term "reference rodent" as used herein, refers to a rodent that is taken for comparison with the rodent of the invention (i.e. the rodent that expresses the human tyrosinase gene in at least the dopaminergic neurons of the substantia nigra pars compacta). The reference rodent can be a wild type rodent (that does not express the human tyrosinase gene) or a rodent of the invention which expresses human tyrosinase, but which is taken at a different developmental stage to the first rodent. The comparison allows defining which biomarkers (metabolites, proteins, etc.) express differentially between both rodents, and can therefore be used in the detection, diagnosis and/or prognosis of neurodegenerative disease.

20

25

30

The term "constitutive" as used herein, refers to a gene that is transcribed (and optionally also translated) continually and does not depend on the presence of an external stimulus in order to give its protein product.

35

The term "inducible" as used herein, refers to a gene that is transcribed (and optionally also translated) only when there is a certain stimulus present in the environment. Thus, an inducible gene is not transcribed continually, as

opposed to a “constitutive” gene. The stimulus can be, for instance, the presence of an effector molecule.

5 The term “Tet-Off” refers to a gene control system where the controlled genes can be activated (expressed) in the absence of tetracycline or one of its analogues (such as doxycycline - dox). In a Tet-Off system, the tetracycline transactivator (tTA) binds to Tet operators (TetO) in the absence of tetracycline thereby activating transcription of the genes under the control of the described operators. In most Tet-Off systems, several repeats of such
10 TetO sequences are placed upstream of a promoter. The coupling of several TetO sequences to the promoter is called a tetracycline response element (TRE), because it responds to binding of the tetracycline transactivator protein tTA by increased expression of the gene or genes downstream of its promoter.

15 The term “tTA” as used herein, refers to a tetracycline transactivator protein of a Tet-Off system, able to bind TetO sequences. In a Tet-Off system, expression of TRE-controlled genes can be repressed by tetracycline and its derivatives, i.e., they bind tTA and render it incapable of binding to TRE
20 sequences, thereby preventing transactivation of TRE-controlled genes.

The term “therapeutic agent” as used herein, refers to any organic small molecule, peptide, protein, DNA, antisense oligonucleotide, small interfering RNA, vaccine, radiation or combinations thereof that could be used to cure,
25 slow down the progression of, or alleviate a neurodegenerative disease. In this particular case, it would modulate the accumulation of NM in order to prevent the initiation of PD or to slow down or halt its progression.

30 The term “transgenic rodent” as used herein refers to a rodent whose genome has been altered by *in vitro* manipulation, with the aim of inserting or deleting certain genetic material with the aim of driving a phenotypic change. In the present invention, the rodent genome is altered at the embryonic stage so that the animal expresses the human tyrosinase gene. This is typically achieved by pronuclear injection of an expression *cassette* into the male pronucleus of
35 purified zygotes and reimplantation of the injected zygotes into pseudo-pregnant mouse females. The transgene randomly integrates into the mouse genome and its expression will therefore depend on the insertion position. In

the present invention, the DNA fragment for microinjection in mice to generate the constitutive transgenic mice is (SEQ ID NO 3):

AGCTTGGCAAGAGCTGATTTTAAAAGGTCCTGGAGAACACAGGCCAGCTCCAGTGTC
AGGCGCCCTCATTCCTCAACTCCAAAGTAATCGCCTGGCAAACAAGCACTGTCTAAATA
5 TCACAGAGTGTGGGTCAGGCGCACCGCAGCCTGGAAATGTCAGTGGCGGGGGCCTCC
TCCCAGCTCCAGGATTAGCCCAGGCACCTGAGCCACATGCATTTGCCTTCAGCCTGG
CTCTAGCCCCAAGAACCCGAAGAGGCCTCAGGTGTGTTCTCACTGCCAGATCGTGGG
ATTGGGTCCCTGGGGAAAGGTGTTGCCCCGTGGGGTCAGACTGAGCCATAAGCGGCTG
TAGCCTTTGGAATGACATACTTCCAAGTTCTCACTAGGAACCCGGAGTAGGGGCAGC
10 CCCGTGGAGAAAGCCTGGCTGTCCCGGGCCCTCACTGCGAGATTACTCACCCCTTGTG
TCCTCCCTTGTCTCTCACTTCTACAAAACCTGACCACTTGCTCAAATGGCAGTGAG
AAGCTGCTGGGTGGGAACTGTTGCTACCATCTGCATGGGCTCTGGGGATGAGGTGGC
CACAGCAGCAGCTGTCATGCTTGTTAGACACAAGGTTCCCTTCACCCCATTAATGGGGC
TGGTGACATTGTCCTAAACAGAAGGGCTTTGCCAAGTAGAAGGATTATTGCTGGGTA
15 AATAAGAGCCCTAAAGGTGGAGTTTTTGTCTTCCAGGAACCTTCTTTAAATCACTAG
GAACTGCCCTTAGGTCAACTGTCTCGGATAAGTGCATCTAGTTTTAGATCAGTCCAA
ACAGCCTCTATCAGTTCAAAGACCCCTACATAAAGTCTGTTGCTCTTCCACCATGCC
CCCTCCACCTCTGAGATAGCCTTGGCAGTTTAATCTCCGATAAACCTTTCTTTCAAC
TCACGGCATGTTTCGTGTTTGGTGGGATCACTGTGGCCTCACAGTAGAGGATGTCCAG
20 AGCTGTGTTTGGCTCTGTCCCTGGACGTTCCCTCTCCCTTGTATTTCGTTTTGTTTTGT
TTTGTGTTTTGTTTTTGTGTTTTTAATCAACCCAACACAACTAGAGTCTTCTTGAAAGA
GGGAACCTTAATTGATAAAATGCCATCTCCAGGCCTAGGAAGATGACTCAACAGTTA
AGAGCACTGGCCACTTTTCCACAGGTCTTGGGTTTGATTCCCAGTCCCCACATGGTG
GCTCACAACCAGTTCTAGTGGATCCAACCTCCCTCTTCTGGCTTCTGCGGACACCAGA
25 CATACAATTGGTATGTGGACACATGTAGGCAAGACATTCACATACATTGAAAACAAA
AAGCCTGTTGAGAGAAAATATCTCCATCAGATTTACCTAGAAGCAAATCTGTATGCA
TTTTCTTGCTTAATGGTTGGTATGAGAGGGCCAGCCCACTGTGGGTGGTAACCATA
GGCTGGTGATCCTGGGGTGCATAGAAAGGCAAGTTGGGAGAGGCACTTTGCAATGTC
CCTTCATAGTCTCAGCTTAAGTCCCTGCCTCCAGGCTCCCACCCTGACTTTTCCCAG
30 TGATGGACAGTGACCAGGACATGTAAGCCAAATAAACTCTCTCCTTCCCAAGTGACT
TTTGGTCCTGGTGTGTTATTACAGCAACAGAAATCAAGCTAGGACACTGCTTTTCTTT
TACTTCATATACTTCGATCATGTGTGTTTGTATGTGATTAGCAGTATGGAAGCCAG
AGGACAGACTAGAATATTGGTTCTTTGATGCATTGGGAGAATTTCTTCCCTGGGGGC
TAGGAGGAGCATGGAGGAGCAGAGACTGGACAACACTTTGGCCTACCAATTGTAGCC
35 CTTCCCTAAGACATCAAGGCCAACTGCAATCAGGGCAGACCATCATAACAGCATGTGG
GGAGGAAGGATTGAGTACTTCAATGAGGGTCCCATGACTCTATCCCCTCCATCCATG
CTGGGCGGTCACTCAGTCAACAAGCCAAGCTGAGAAATCCTCTGCAGGCTTAACCTCAA

GCGAAGTCATGAAGTCAAGATAATTGGTTGTTTTGGCTTGAGAATAGTGCTATGTAAAC
AGCCGAGTCCACCTTGCCCTTGAGACCATATCCCACACTGGCCTGGAGCTCGCCAGGT
AGGCTACACTGCTTTTAGCTGAGTCCTAGGGATCCTCCTGCCTCTGCCCTCCTCACTG
CTGGGATTAGAAAACATGCACCACCACACCTGTCTACATGTCTTCTAGGAATCAGACT
5
AGGCCCCATATGCTGTGTAAAAAGAACTCTGTGGACTGAGTTACCTCTGTACACTACA
GAGCAAGTATTTAACTGGGGGCTGGCTTACAATTTTCAGAGGCTTAGTCCATTACAAT
AAGGGCAGGGAGCATAGCAGTGTGCAGAACTCTGAGAGCTACATCCTGATCTGGAGAG
AGAAAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGACAGA
CAGACAGACACTGAACCTGGCATGGACTTTTAAAACCCAATAACATGCCCCACTCCA
10
GCCAGGCCACACCCACTCTGACAAGGCCACAGTTCCTAACACTTCTCAAATAGTGCC
ACTCCTCAGTGACTACGCATTCAAACACAGCAGCCACACTAGGTA ACTATGAGGCCG
CAAAGCCACGGGATGGGAGCCCTCTCCACCTGGAGAAGCCTACCCTAATGACAGAGG
TGATAGGGTTATTTCTCCTGAGTTCCCCAGACTGGACCCCAACACATTAAAGTCTG
CTCTAAACCAAGCCAAGCTGCCCCCTGGGCCTCCCCTGCCAATGTCCTCCACACATGC
15
AGAAGCTACACCTCCTTTGGACATCTCTTCAGTTCAGGGGGACAAAGCTCCAGAGTC
TGCTGCTGTCCCATCTCCTGTCCCTGCCCCATAGGCATCCTCCATAGGCAGGGCCCTA
GAAGTTTTTCTACTGTGGCTTGGCACATGTGAGGCAAGTGAGACTGTAAGACTATCC
CTGCTAAATGCTGGGGCCACTGTA ACTCTGCTAACACAGCCCCGGGCCATGCTGTGG
GGAGCAGAAGCCACCTTGTAGATGAGAAGAGAGGACTATACACCACCACAAACTGCA
20
AGAAAGAAAGCACTCCCATTTCGGAGCAGGAACCACTGCCCCCTCCCTACCACAGGATG
GGAGGGGGGACCACCACCCACAGGCTCCAGTGCTTCCGTGGGCCTGGGGTGGGTAGGC
AGCCGTCTTGCAATTAGACATGATTGCTGTCACATCACCACCGTGGTCCTTGTGAGT
GACTAATGGGA ACTGAATGTCACTCTTATTGCTTTTACTGGCTAATTTGTCAGACTG
TCAAGTACTCTGGGAGGTGGCACGGAGGGTATTACGTGACTCACTGTGCACAGGTTG
25
GGAGAGGCAAGGGCCTTAGGGGGGACAGAGGGAAGCAGGAATACCAGAGAGGACCAG
GGGTCTGACCCATGACATTTTCGGAAAGTCTGAGGGGCATGGGAGCTGAGGTTACCC
CACCTGTCACATATCGATGGCTCTGAGTTCCTCTAGGCCCTTCTGTGTACACACATG
CTTATATGAAGGCTTGGGGATATAGAACACATGCCCTGCCCTGCATGTGCATATAGA
CACAATGGCATGTGCATAAGGCATGTACATGTGCATGCACATGTGCACATACATGTA
30
GTGTATTGCATT CAGACCAAATGAGGTTAGATATAGATCATGGGTCCCTGGCCTGGA
CACTCATGTAGCCAGCCACTAGTGAATTTGGCATGACACCTCTCTATCATCTCAGGG
TTGGTGTGTAGGTTGCCAGGTGGTGTGGGTTGCCAGGTGACATAGGTGTAGCTGTG
AGCCTCTGCTAATGTGAGGCTAGTGTTGAGCGCTTCAGAGAAGCCTGGTTCTAATGC
TGACTTCCTGTTGGCTAGGTCTAACAGAGGGACAGGCTCTCTAACCTGGACTCACTC
35
ACTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
CTGTCTCTCTGTCTCTGTCTCTTGCCTTAGTTGCACTCTGGCTATTGGTCCTCCC
TCCTGGTGACCACCTGACCCAGGCCCTTGGCCTTTGAGAACGCTACACACCTAAC

TAAAGTCATCTCCTACTAGAAAGCTCAGAGAAGGGCCAGCCATCTCTCAGCAGATGGA
GAGTTCTTTAGAAAGCACCAGAGCTCCTGAGAGCTGCCACGAGTCACTCCCAGGAATC
ACCGTGAAGCTAGGGAAATCAAGGCACAAGGTACGAGAGGCTGAGCCACGCTGGGGC
TCTCTTACCTGTAGACAGTGAATCTCCAGGAATAAGGCAGGCAGAGAAGCAGTGCTG
5 GTCTTACTAGCCAAAGGTGGAAGTGGTTTAGGGGAGCGAGGAGGGTGGGCTGCCTGG
AAGATACTCTGGACCTGAAGGCTTGGTGGAAAGTGGGGAGCCAGGGTAGAGGAAGCAG
ACAGGTGGGGGCTCCTTGGGGAGAGAGGGCCTAGAGCCTAGGTTGAGACGGGGGCTG
ATCAGCAGCTCTTGCCTCTGGTCTGACTTTCAACTGCCCAATTATCCCTAAGTGTCT
CCTATCGACCGACCGCAGCTGGTGCTGTCCGGTACGTGTTCTGAGTCTACAGCCCCG
10 AGGCTGCTGCTCCATCCGATGGCCTCGTTAGGGCTAATTGCTCTGGCATTGTTGGCCT
GATGAGGACAAGAATGGCTGGACCCTACCAGATGTCAGGGAGCTTCATTCTCTTTCC
AGTCAGATTGGTGAGCTGTGTCAGGGACAAAGGGCCCTGCGGTCTCACTGAGCATCA
GGTCCCTGCTTTACAACCCATCTCAGTCTCCTGGTGGCAGGGAAAAGCAACCTCACT
CTCCCTTCTGTCTTGGGCTCACCTGCACAGCACTTAGGCAACTGGTCATGATGTCT
15 TGACCAACTTAAATCCAAGAGAAGCTCCCCTGCGTTTCAGGGTATGTCAGTTGATG
ATTTTCATGGACCTGCATCTCTGTGAGCAGGAACACTGACCCCAGAGCCCATCTGGTG
ACAGGATTCTTGAGACCCTGGCATCATGTGTTTCTATAGAAAGCGATTTGGGTGGAC
TTGTCAAAGTGGGTGGGGTATGTGTTTGCCTGTGTGTGTGTGGGGTCTATAGGTAT
AGCATGTTTGTGAATGCATGAACATATTATAATACATGTATGCCTATGCGATTCTGT
20 TTGTATGTGTATATCTGTGTTTCGCATATGTTTGTGTGTGCATGTGGCTGCTCCTATG
TATACTTGTGTATGTATTTGTGTGTCTGTGTCTCCATGTATAAGCACTGTGAATCTG
TATATCTGTATGTGCATACGTTTGTGTGTCTATGTGTCTATGTATATAACCATTTGTGT
ATATACATTTGTGTGTGCCAATCTCTCTCTGAATGTATCTGTATATGGCCATATGTG
TACATGTATGTCTTTATACATGTGTGTATGCCTATGTATGTCCTCATATATGTGTGG
25 TCATGTGTCTCTGTGTGTACATGCTTCTGCGTGTCTATGGTAGAAGGTCATGATGCT
GGTTGAAAGTGGCCTTTGAGCCTAGGTTGTAGAGGCATGTGTTCCAAAATGCACATA
GGTTTCCATAGTGCCCAAAGCTATGGGGTAGATCTGGGGTCCTTGCTCCAAGACCAA
GGATCAGGACACCCCTAGTCCTCTGCCTCCTTAGTTCCATGATGTCCTGGGCAGGG
AGGTCCATATCAGCAGGGTGCATACAGGATAGCACAGCAGCCTCCACACCAGCAACA
30 CTGATGCCAGGGAGTGGGTGAGCTGTCATTATGGACAATTAAGCACCGTGCTGAGAG
AGAAAGGCCCGTGTGCTCCCGTCTCTGCAGGCCTAATCGCTGTGGTGACAGAGGA
GGGTAATTATCAGGACGGCTGTGAATGGCACAGGCATTACAGGGGTGTAAAGGGCTC
AGGAAGGAGGGAGGGCCTGCTATGCCCCAGCACGCATCTGAGCCCAGAGCAGGCAA
TGGCCCACTGCACTGGCCTTGCGCTTCAGATCAGCCCCCTTTCTTAACTGCTAGGGG
35 ATGCTTCCCAATCACTCCTCTAGGCTCTGCGGCTTGCTTCCAGCCTGTACGCTGTC
CAGAGAGCCTTCAAAGCCTCACTTCGACCAACCAGAAGCCTCTCGTCAGCCCTGCCC
TGACCTCGTGTGCCTCTTCAAAGTGAGATTTAGCAGCTGCAGCTGGGGGTGCCTGAG

CCCCACTCATGCTGTCTTCCTTGAAGACAGAAGTGTGTTGGGAGCTGAGGACCTGGGCC
GTATGATCCAGAGAAGTAGTGTGCTTCTGGGTCTCAGCTCTCCCTTCTGCAGAATGG
GTCTGTCTGAAATGGAAAGGCAGGTGCCCCCTCTGCAGGGCCTAATCTGAGTCGCCAT
GAGTGGTTAAAAGATCCAGCTTGTCTGTGGGTGAGCTTTGAGAGGAGGCAGGGACCT
5 CTAGCATGGAACAGGGCTGAGTCCTGGAAAGCTGACCAAGGGCAGGCCTAAGAGGCC
TCTTGGGATTCTTCTCATCAAAAAGGGCATGGGACACAGCTAAAGCGTCCAGGGCTC
CTCTGTGCCCACAGATGCCTTAGATCTTGGCACAATGTAGTCAGCCAGCTCCGTGTG
TGTGTGTGTGTGTTTGCATGTATCTCACAGACAGTGCACAATGGCCTGGATGTGAAC
AGAGGCAAGAGTCTGGGCCAGCAGTTGTCTCCCAGGAGGGTCCAAAGACATCGTATT
10 TTCAAGTTTAGGCCAGGTGCTCACTTGGGTGAGCTCAGACACAGACAAAGGTCTGGA
GAGCACACATTCCCCACCCCCACCCAGCTCCTATGCAAGCACCTCCAGCCGAGACAA
GAAAACGAATTAAAAAGCAATATTTGTGTGTCAGCGTAAGACATTTGCCGAAAGGTAA
ATCCACACTCGTGGTGCTGCACAGCAGCCCCCTGTGCAGGATTTGTTAGGCACAGCT
CCCTCCTACCCCGTGCCACCTGAGCAAATGCCAGGCTGGGTGGGCTGGAACCAGCCT
15 GGGCTTGCCTCACCTGGAATCCCCAGCACCTCCAAAGGAGGACCCTGGGAGTGGG
CATAGACGCCCTTCAGGTGTGGGCAACAGCCCCCAGTCCTCAGGATGAAAGGCTAAG
GTGCAGCCAGCTCTGCCTTCACGGTGGGAATGTCTCTATGTGAGCCCTTTCTGGGCT
GTGAAGAACGCTCTGAGAAGGGTCCTGGGACCCTGGATAGGCCAGAGCTGTGCTGGG
CATGTAGAGACAGGAGTGGGCTAAAGCAGCAAAGGCACTGACCAAGGAAGAGTTTCAG
20 AGAGGAGCGTGGAATATGGGGAGGGGTTTCATAGTAAGAGAGAGCAGGCAGTGGAGAG
TAAATAGTCACTGAGCCGGGGTTTTATGGGGTTTTGTAGGAGCTTACTCAGAGAAAGTA
GATGAGAGATGCCATGCCAGTCTGAGTATCACAGAGCCCCAGGCTCTCCTGGGAACG
GAACTGTGAGGGCCAGAAGGTCAGCAAGGGAGGTTAGGGAGAGTTCTTTTGTACTG
ACTCAGCATTTATCCTGCTCCCAGGGGGCAATGGGGGCCAGTGAGGGATGCAGAGCA
25 AGGCAGTGATGTGGCAGGCAGTTCTGTGTGAAAGAGCTGGGAAGGGAGCGGGCTG
GGCCTGGTACGTACAGCAGGCCATTTCTGAGGGTCCGAGTGCTGTCTAGGAGGTGCA
GTGAGACTTCAGTGATCAGCCAGAACAGAAGCTAAGCGGGGTGGGGACTGCGAGTTC
AGGCTTCTGGGTCTTGCAAATATCCAGAATGCTAAATCCTCAGAACCCCAGGGTGGC
CATTTTCAGAGTGGGTTTTGTCTTTGGGCACCTTGTGCAGACTCCAATATCCAGAGG
30 GATAAGGATGGTACTCTTCAGTACCCTTAGTGAGAGGACACTTTTCTCTGAAGGGCT
TGAATGTGCCGAGCCATTACCTGAAGGAAGGAAATGACTCCAGGGACATAGGATGGG
CCCAGCACAACTCACCTGCTACAGAGAAAGGTCCCCCTCCCTGGTCTCCTTAGAGATC
CTGTTTCCCTGGCTGAGGAAGCTAGGGTGGATCTTTGTGTAAAGTGGGTGTGGATGCT
AACTGGAAAACAAAAGGTCACCTTACTGTTAGACCTCGGGGTACCATGGAAGAGATGA
35 TCACTGAGTGTGCCCTTACATGGGGACCAGCTGAGAATGGGGCTACCACTAGCTCGA
GACCATGATACAGGGAATAAGTGTGCATTTGGGGGTAGGGAGTGGCTCAGAATACTC
TTAACCAAAGCAGAGGTTTTGCTCCCACAGGAAGGTGAGGTCAGAAGGCCTTAGGGAG

CTGCCAGGGGCTAGGGTTGGCACCATCTCCCAGGCTGTGTCTTTAAGGAGATGATAA
TCAGAGGGATAGAACCTTGCAAAAGTGGGCCAGTCTTGGGAATACTATAGAGGAATA
GCCTTCTGGAACATTCTGTGTCTCATAGGACCTGCCTGGGGATCCAGCCCCAGTGCC
AGCACATATAACCGACTGGGGCAGTGAATAGATAGTACACTTTGTTACATGGGCTGGG
5 GGGAACATGGCCCATGTCCTGGAGGGGACTTTATGACAGACATCCAAAAATCCAGTG
AGAGGGCTTCTAGATTTGTCTCCAAAGGTTATAGTTCTAACATGAGCCCTTAGGAAA
TCCAGCATGGTTCTCCCTGTGTGCCCTGGTTTGGTTAGAGAGCTCTAGCGGTCTCCT
GTCCACAGAATAACCAGCCAGCCCCCTGCCCTACGTCGTGCCTCGGGCTGAGGGTGAT
TCAGAGGCAGGTGCCTGTGACAGTGGATGCAATTAGATCTAATGGGACGGAGGCCTT
10 TCTCGTCGCCCTCGCTCCATGCCCACCCCCGCTCCCTCAGGCACAGCAGGCGTGGA
GAGGATGCGCAGGAGGTAGGAGGTGGGGGACCCAGAGGGGCTTTGACGTCAGCCTGG
CCTTTAAAGAGGGCGCCTGCCTGGCGAGGGCTGTGGAGACAGAACTCGGGACCACGa
attctgcagatatccagcacagtggcgccgctcgaccGACCTTGTGAGGACTAGAG
GAAGAATGCTCCTGGCTGTTTTGTACTGCCTGCTGTGGAGTTTCCAGACCTCCGCTG
15 GCCATTTCCCTAGAGCCTGTGTCTCCTCTAAGAACCTGATGGAGAAGGAATGCTGTC
CACCGTGGAGCGGGGACAGGAGTCCCTGTGGCCAGCTTTCAGGCAGAGGTTCCCTGTC
AGAATATCCTTCTGTCCAATGCACCACTTGGGCCTCAATTTCCCTTCACAGGGGTGG
ATGACCGGGAGTCGTGGCCTTCCGTCTTTTATAATAGGACCTGCCAGTGCTCTGGCA
ACTTCATGGGATTCAACTGTGGAACTGCAAGTTTGGCTTTTGGGGACCAAACCTGCA
20 CAGAGAGACGACTCTTGGTGAGAAGAAACATCTTCGATTTGAGTGCCCCAGAGAAGG
ACAAATTTTTTGCCTACCTCACTTTAGCAAAGCATAACCATCAGCTCAGACTATGTCA
TCCCCATAGGGACCTATGGCCAAATGAAAAATGGATCAACACCCATGTTTAACGACA
TCAATATTTATGACCTCTTTGTCTGGATGCATTATTATGTGTCAATGGATGCACTGC
TTGGGGGATCTGAAATCTGGAGAGACATTGATTTTGCCCATGAAGCACCAGCTTTTC
25 TGCCTTGGCATAGACTCTTCTTGTGTGCGGTGGGAACAAGAAATCCAGAAGCTGACAG
GAGATGAAAACCTTCACTATTCCATATTGGGACTGGCGGGATGCAGAAAAGTGTGACA
TTTGCACAGATGAGTACATGGGAGGTCAGCACCCCAAAATCCTAACTTACTCAGCC
CAGCATCATTCTTCTCCTCTTGGCAGATTGTCTGTAGCCGATTGGAGGAGTACAACA
GCCATCAGTCTTTATGCAATGGAACGCCGAGGGACCTTTACGGCGTAATCCTGGAA
30 ACCATGACAAATCCAGAACCCCAAGGCTCCCCTCTTCAGCTGATGTAGAATTTTGCC
TGAGTTTGACCCAATATGAATCTGGTTCATGGATAAAGCTGCCAATTTTCAGCTTTA
GAAATACACTGGAAGGATTTGCTAGTCCACTTACTGGGATAGCGGATGCCTCTCAA
GCAGCATGCACAATGCCTTGCACATCTATATGAATGGAACAATGTCCCAGGTACAGG
GATCTGCCAACGATCCTATCTTCCTTCTTACCATGCATTTGTTGACAGTATTTTTG
35 AGCAGTGGCTCCGAAGGCACCGTCTCTTCAAGAAGTTTATCCAGAAGCCAATGCAC
CCATTGGACATAACCGGGAATCCTACATGGTTCCTTTTATACCACTGTACAGAAATG
GTGATTTCTTTATTTTCATCCAAAGATCTGGGCTATGACTATAGCTATCTACAAGATT

CAGACCCAGACTCTTTTCAAGACTACATTAAGTCCTATTTGGAACAAGCGAGTCGGA
 TCTGGTCATGGCTCCTTGGGGCGGCGATGGTAGGGGCCGTCCTCACTGCCCTGCTGG
 CAGGGCTTGTGAGCTTGTGTGTCGTCACAAGAGAAAGCAGCTTCCTGAAGAAAAGC
 AGCCACTCCTCATGGAGAAAGAGGATTACCACAGCTTGTATCAGAGCCATTTATAAA
 5 AGGCTTAGGCAATAGAGTAGGGCCAAAAAGCCTGACCTCACTCTAACTCAAAGTAAT
 GTCCAGGTTCCCAGAGAATATCTGCTGGTATTTTTCTGTAAAGACCATTTGCAAAAT
 TGTAACCTAATAACAAAGTGTAGCCTTCTTCCAACCTCAGGTAGAACACACCTGTCTTT
 GTCTTGCTGTTTTCACTCAGCCCTTTTAAACATTTTCCCCTAAGCCCctagagggccc
 gtttaaaccgcgtgatcagcctcgactgtgccttctagttgccagccatctgttggt
 10 tgccccctcccccgctgccttccttgaccctggaaggtgccactcccactgtcctttcc
 taataaaatgaggaaattgcatcgcatgtgtctgagtaggtgtcattctattctgggg
 ggtggggtggggcaggacagcaagggggaggattgggaagacaatagcaggcatgct
 ggggatgcggtgggctctatggcttctgaggcggaagaaccagctggggctctagg
 gggtatccccacg

15

As mentioned above, a first aspect of the invention is a rodent that comprises the human tyrosinase gene, the gene being expressed in at least the dopaminergic neurons of the substantia nigra pars compacta.

20 In a particular embodiment of the first aspect of the invention, the rodent progressively accumulates NM in said neurons along its lifetime.

In a particular embodiment of the first aspect of the invention, the human tyrosinase gene is the human tyrosinase gene of SEQ ID NO 1.

25

In another particular embodiment of the first aspect of the invention, the human tyrosinase gene has been transduced with a viral vector. Thus, the rodent is a rodent transduced with a viral vector comprising the human tyrosinase gene.

30

The former embodiment can be reformulated into a method of obtaining the rodent of the first aspect of the invention, comprising transducing the human tyrosinase gene via a viral vector which is injected into the brain of the rodent.

35 In another particular embodiment of the first aspect of the invention, optionally in combination with any of the embodiments above or below, the viral vector is an adeno-associated viral vector.

In another particular embodiment of the first aspect of the invention, the adeno-associated viral vector, is the adeno-associated viral vector of SEQ ID NO 2.

5

In another particular embodiment of the first aspect of the invention, the rodent is a transgenic rodent.

10

In another particular embodiment of the first aspect of the invention, in the transgenic rodent, the expression of the human tyrosinase gene is constitutive.

15

In another particular embodiment of the first aspect of the invention, in the transgenic rodent, the human tyrosinase gene is operably linked to the promoter of a rodent tyrosine hydroxylase.

20

In another particular embodiment of the first aspect of the invention, the transgenic rodent departs from an embryo that has been injected with the construct comprising SEQ ID NO 3. In particular, the construct is the construct consisting of SEQ ID NO 3.

25

The former embodiment can be reformulated into a method of obtaining the rodent of the first aspect of the invention, by using the following protocol: a) designing a gene construct comprising SEQ ID NO 3; b) microinjecting the gene construct in the masculine pronucleus of a rodent fertilized ovus; c) culturing the obtained ovus; d) transplanting the latter into the oviduct of a female pseudopregnant rodent; e) selecting, among the offspring, those animals which contain the cDNA coding for the transgene.

30

The former embodiment can be reformulated into a method of obtaining the rodent of the first aspect of the invention, by using the following protocol: a) designing a gene construct consisting of SEQ ID NO 3; b) microinjecting the gene construct in the masculine pronucleus of a rodent fertilized ovus; c) culturing the obtained ovus; d) transplanting the latter into the oviduct of a female pseudopregnant rodent; e) selecting, among the offspring, those animals which contain the cDNA coding for the transgene.

35

In another particular embodiment of the first aspect of the invention, the expression of the human tyrosinase gene is inducible.

5 In another particular embodiment of the first aspect, the transgenic rodent is an inducible double transgenic rodent derived from mating a first parent rodent comprising the gene coding for the tTA transactivator operably linked to the promoter of a rodent tyrosine hydroxylase, and a second parent comprising the gene coding for the human tyrosinase operably linked to a promoter under the control of the target sequence for the tTA transactivator.

10

The former embodiment can be reformulated into a method of obtaining the rodent of the first aspect of the invention, by using the following protocol: a) generating a first parent rodent comprising the gene coding for the tTA transactivator operably linked to the promoter of a rodent tyrosine hydroxylase; b) generating a second parent rodent comprising the gene coding for the human tyrosinase operably linked to a promoter under the control of the target sequence for the tTA transactivator; c) mating both parent rodents to obtain the double transgenic rodent.

15

20 In another particular embodiment of the first aspect, optionally in combination with any of the embodiments above or below, the rodent is selected from the group consisting of rat and mouse.

25 As also mentioned above, a second aspect of the invention is a method of screening for therapeutic agents that prevent, delay or halt the development of neurodegenerative disease in the rodent of the first aspect of the invention, comprising: 1) exposing said rodent to at least one candidate therapeutic agent; and 2) assessing the effect of the at least one therapeutic agent on the progression of neurodegenerative disease in said rodent.

30

In a particular embodiment of the second aspect of the invention, the neurodegenerative disease is associated with neuromelanin accumulation.

35 In another particular embodiment of the second aspect of the invention, the neurodegenerative disease is associated with α -synuclein accumulation.

In another particular embodiment of the second aspect of the invention, the

neurodegenerative disease is PD.

Throughout the description and claims the word "comprise" and variations of the word, are not intended to exclude other technical features, additives, components, or steps. Furthermore, the word "comprise" and its variations encompasses the term "consisting of". Additional objects, advantages and features of the invention will become apparent to those skilled in the art upon examination of the description or may be learned by practice of the invention. The following examples are provided by way of illustration, and they are not intended to be limiting of the present invention. Furthermore, the present invention covers all possible combinations of particular and preferred embodiments described herein.

EXAMPLES

A) Material and Methods

Generation of vectors

The pcDNA4_TYR construct containing the coding sequence of the human tyrosinase gene (TYR, 2.082 bp) (Hasegawa et al. "Increased dopamine and its metabolites in SH-SY5Y neuroblastoma cells that express tyrosinase" J. Neurochem. 2003, vol. 87, pp. 470-475) was used as a parent construct to generate the adeno-associated viral (AAV) vector and the transgenic mice.

To generate the AAV2/1-CMV-Tyr (SEQ ID NO 2), the human TYR was subcloned from the pcDNA4_TYR construct into an AAV of serotype 2/1, commonly used to transfect distinct areas of the brain. The AAV encodes an internal ribosome entry site (IRES) under the control of cytomegalovirus immediate-early (CMV-IE) promoter and contains a SV40 polyadenylation signal, flanked by two inverted terminal repeats (ITRs). CMV-null AAVs of SEQ ID NO 4 were used as control (its sequence found below). AAVs were generated and purified by the Viral Vector Production Unit (UPV) of the Center of Animal Biotechnology and Gene Therapy (CBATEG) at the Universitat Autònoma de Barcelona (Barcelona, Spain). Titers obtained were in the range of 1.2×10^{12} to 2.5×10^{13} vector genomes per ml (vg/ml).

SEQ ID NO 4:

CAGCAGCTGCGCGCTCGCTCGCTCACTGAGGCCGCCCGGGCAAAGCCCCGGGCGTCGG

CGGACCTTTGGTCGCCCCGGCCTCAGTGAGCGAGCGAGCGCGCAGAGAGGGAGTGGCC
AACTCCATCACTAGGGGTTTCCTTG TAGTTAATGATTAACCCGCCATGCTACTTATCT
ACGTAGCCATGCTCTAGACATGGCTCGACAGATCTCAATATTGGCCATTAGCCATAT
TATTCATTGGTTATATAGCATAAATCAATATTGGCTATTGGCCATTGCATACGTTGT
5 ATCTATATCATAATATGTACATTTATATTGGCTCATGTCCAATATGACCGCCATGTT
GGCATTGATTATTGACTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATA
GCCCATATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGAC
CGCCCAACGACCCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGC
CAATAGGGACTTTCCATTGACGTCAATGGGTGGAGTATTTACGGTAAACTGCCCACT
10 TGGCAGTACATCAAGTGTATCATATGCCAAGTCCGCCCCCTATTGACGTCAATGACG
GTAAATGGCCCGCCTGGCATTATGCCCAGTACATGACCTTACGGGACTTTTCCTACTT
GGCAGTACATCTACGTATTAGTCATCGCTATTACCATGGTGATGCGGTTTTGGCAGT
ACACCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTCCAAGTCTCCACCCCA
TTGACGTCAATGGGAGTTTGT TTTGGCACCAAAATCAACGGGACTTTCCAAAATGTC
15 GTAACAACTGCGATCGCCCGCCCCGTTGACGCAAAATGGGCGGTAGGCGTGACGGTG
GGAGGTCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCACTAGAAGCTTTAT
TGCGGTAGTTTATCACAGTTAAATTGCTAACGCAGTCAGTGCTTCTGACACAACAGT
CTCGAACTTAAGCTGCAGTGACTCTCTTAAGGTAGCCTTG CAGAAGTTGGTCGTGAG
GCACTGGGCAGGTAAGTATCAAGGTTACAAGACAGGTTTAAGGAGACCAATAGAAAC
20 TGGGCTTGTCGAGACAGAGAAGACTCTTGCGTTTCTGATAGGCACCTATTGGTCTTA
CTGACATCCACTTTGCCTTTCTCTCCACAGGTGTCCACTCCCAGTTCAATTACAGCT
CTTAAGGCTAGAGTACTTAATACGACTCACTATAGGCTAGccagcacatgactgaca
ctcggaagcacagaacaaagggtaggacggtgccccatgggtcaggctgtagccacgc
caccctttccaccctgtcctagccagaggcagcaatgtgctccatacagatcctcct
25 aacacacccacactgtcgggtccccagcacgcagatgccccgacagccccttaggcaaa
tggcttagctgactgccccaccacacgcgcgtcgccatgcagtccagtggggagtcgg
aggcagcctccttcctgcctctcctcggcctgcacgtgtccccccaccaggcagaga
cccttctacacccccgggtgtctgcgggtcacatcgcggtggggcatgcagctgttggc
cttcgagcatgttttg ttttccttggccagtgctctccagagaaacgcacgtgggttt
30 gtgtccagcgggtccatctctgcaacagttgttcctttgggattggatgctaggaggt
cacgggagaggtgtccatccaaagcagtgctctgtgtcacacactgtccccacacaca
gggccacctctgcacagactcccccgactcgattctgggcacagagctcagtgacct
tccagagactgccacgaaccgggtgatgcctccacgcttgagacatcctgaccgcagg
gccaaggcgcactgggtcaggggggtgacagtgaggggtctgcaaacagactgctga
35 tgctcaacccggccgctgccgagctgtgtgacttgggcacgtcacttaacctctctc
ggcctctgtctcctcccggggataagagtagtagcacctgcttcccggggctgtgag
gatccagtggggacgtataggaactagcgaggcaccggcagttgggtcagagctactg

ttgtcacttcacaaggcatttttcttcaacagcaagtcggaaatctcatgagcctaag
gcagaatccacctgtggcctctggttacaacccacaggactgaaaatccttccagcc
acagcaactggtgaatttcctggtcaattgccacaagtcatgagctgaaccccactt
gagtttcagttcaggcagaactctagagacgactagggcaagctagacagcgactgc
5 agagccttttgttgacgctgagcagtcctcagctggttgacatcactggggagcaaa
cgaggaccaggagcggtgaaaggacagtgtctgctgcagattgtcgtagcacccaag
gaacactccagaaagcctcctaagcagtaacaagtggtggaagggtgtagcccagcca
acagtggcatctgcgaggcgctcccctccttccctcccactaccccgatataccctggga
cctgtgcactgaaggactcattctaaaggctgtgcccctgcagccgccagcctcact
10 cactggctgcctgtgccagctagagatttcttctcctctgaggctggctgagaggacc
actccagtttctggtgcccacccagcaaaagaagatacacatcatgcacgtgtaaaatg
aggaaccggtttattgaacagcttaaggagagcaaaaaatagtggcttttagctacatt
ttttacacactgagcaggaaagtctaaaccatcccgttcccctgtaccccaaagaga
acagggcttgctggaggccagtgccaagggcgagtcgtgctcgcagcagacttgaa
15 ttaaccccatgtagggccggcgagcagttgcccgcgtgaaaacaccaccctcttctcc
tggctgagaagatcaaagctctttttttaccctcttttccagcaaaggacctatttgt
tttcaggcaggaggatgttaaacttgacgctctgacacacgggtggaacctgcagtg
cttgagaaaacggcacgcacacgtgaaaacatcatgcctactccaaagccttcttgt
tgctggcaggagggaagcttgagactttcccacgcatagtcgtgaccgcgctggccg
20 tttctgctctcagcaacattctctagtgttccggcttcaagcagcgcttgctcaggtt
tgaagctagcctcgacctcgagacgcgtgatatcggatcccggccggcgccgcTTC
CCTTTAGTGAGGGTTAATGCTTCGAGCAGACATGATAAGATACATTGATGAGTTTGG
ACAAACCACAACCTAGAAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGTGATGC
TATTGCTTTATTTGTAACCATTTATAAGCTGCAATAACAAGTTAACAACAACAATTG
25 CATTCATTTTATGTTTCAGGTTTCAGGGGGAGATGTGGGAGGTTTTTTAAAGCAAGTA
AAACCTCTACAAATGTGGTAAAATCCGATAAGGGACTAGAGCATGGCTACGTAGATA
AGTAGCATGGCGGGTTAATCATTAACTACAAGGAACCCCTAGTGATGGAGTTGGCCA
CTCCCTCTCTGCGCGCTCGCTCGCTCACTGAGGCCGGGCGACCAAAGGTCGCCCAC
GCCCCGGGCTTTGCCCCGGGCGGCCTCAGTGAGCGAGCGAGCGGCCAGCTGGCGTAAT
30 AGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAA
TGGAATTCCAGACGATTGAGCGTCAAAATGTAGGTATTTCCATGAGCGTTTTTCCGT
TGCAATGGCTGGCGGTAATATTGTTCTGGATATTACCAGCAAGGCCGATAGTTTGAG
TTCTTCTACTCAGGCAAGTGATGTTATTACTAATCAAAGAAGTATTGCGACAACGGT
TAATTTGCGTGATGGACAGACTCTTTTACTCGGTGGCCTCACTGATTATAAAAACAC
35 TTCTCAGGATTCTGGCGTACCGTTCCCTGTCTAAATCCCTTTAATCGGCCTCCTGTT
TAGCTCCCGCTCTGATTCTAACGAGGAAAGCACGTTATACGTGCTCGTCAAAGCAAC
CATAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCA

CGGTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTT
CCTTTCTCGCCACGTTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTT
TAGGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCCAAAAAATTGATTAGGGTG
ATGGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTTCGCCCTTTGACGTTGG
5 AGTCCACGTTCTTTAATAGTGACTCTTGTTCCAAACTGGAACAACACTCAACCCTA
TCTCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAA
AAAATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAAATATTAACGTCTA
CAATTTAAATATTTGCTTATACAATCTTCCTGTTTTTTGGGGCTTTTCTGATTATCAA
CCGGGGTACATATGATTGACATGCTAGTTTTACGATTACCGTTCATCGATTCTCTTG
10 TTTGCTCCAGACTCTCAGGCAATGACCTGATAGCCTTTGTAGAGACCTCTCAAAAAT
AGCTACCCTCTCCGGCATGAATTTATCAGCTAGAACGGTTGAATATCATATTGATGG
TGATTTGACTGTCTCCGGCCTTTCTCACCCGTTTGAATCTTTACCTACACATTACTC
AGGCATTGCATTTAAAAATATATGAGGGTTCTAAAAATTTTTATCCTTGCGTTGAAAT
AAAGGCTTCTCCCGCAAAAGTATTACAGGGTCATAATGTTTTTGGTACAACCGATTT
15 AGCTTTATGCTCTGAGGCTTTATTGCTTAATTTTGCTAATCTTTGCCTTGCCCTGTA
TGATTTATTGGATGTTGGAATCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGC
GGTATTTTACACCGCATATGGTGCCTCTCAGTACAATCTGCTCTGATGCCGCATAG
TTAAGCCAGCCCCGACACCCGCCAACACCCGCTGACGCGCCCTGACGGGCTTGTCTG
CTCCCGGCATCCGCTTACAGACAAGCTGTGACCGTCTCCGGGAGCTGCATGTGTCAG
20 AGGTTTTTACCGTCATCACCGAAACGCGCGAGACGAAAGGGCCTCGTGATACGCCTA
TTTTTATAGGTTAATGTCATGATAATAATGGTTTTCTTAGACGTCAGGTGGCACTTTT
CGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATG
TATCCGCTCATGAGACAATAACCCCTGATAAATGCTTCAATAATATTGAAAAAGGAAG
AGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTTCGGGCATTTTGC
25 CTTCCTGTTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAG
TTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAG
AGTTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGT
GGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCGCATACAC
TATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGAT
30 GGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAAAGTGC
GCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCAC
AACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCC
ATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGC
AACTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAAGACTGG
35 ATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGG
TTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCA
CTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAG

GCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAG
 CATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAACTT
 CATTTTAAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACCAAA
 ATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAA
 5 GGATCTTCTTGAGATCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAA
 CCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCG
 AAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCG
 TAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTA
 ATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGAC
 10 TCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCTGGGCTGAACGGGGGGTTCTGTC
 ACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAG
 CTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGC
 GGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTAT
 CTTTATAGTCCTGTCTGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGC
 15 TCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCCTTTTTACGGTTC
 CTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCT
 GTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACG
 ACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAACCG
 CCTCTCCCCGCGCGTTGGCCGATTTCATTAATG

20

To generate tissue-specific transgenic rodents, the 9 Kb upstream rat tyrosine
 hydroxylase (TH) promoter was cloned into the pcDNA4_TYR construct using
 the restriction sites HindIII and EcoRI. The TH_pcDNA4_TYR construct was
 then amplified in XL1-Blue bacteria and isolated using the NucleoBond Extra
 25 Midi Kit (Macherey-Nagel). 10 µg of construct DNA were sequentially digested
 with the enzymes HindIII and BciVI and run overnight in a 1% agarose gel
 electrophoresis to isolate the 11 Kb DNA fragment to be injected. This
 expression cassette, a lineal DNA fragment containing the rat tyrosine
 hydroxylase (TH) promoter, the human TYR gene and a polyadenylation site,
 30 was purified by electroelution.

To obtain inducible rodents two different constructs are required: (i) parent
 construct pcDNA4_TYR containing the TYR gene and a tetracycline-
 responsive element (tet-O); (ii) a tetracycline transactivator (tTA) construct
 35 (pcDNA6/TR_TH) containing the rat TH promoter. The expression cassettes
 are excised by enzymatic restriction and purified by agarose electrophoresis
 followed by electroelution.

Stereotaxic injection of the AAV-Tyr vector.

Overexpression of human tyrosinase (Tyr) was induced by unilateral stereotaxic injection of 2 μ l of the AAV2/1-CMV-Tyr (1.70×10^{13} vg/ml) into the substantia nigra (SN) of adult Sprague-Dawley rats [stereotaxic coordinates: -5.2 (AP), 2 (L) and -7.6 (DV)] or 1 μ l in C57BL/6 mice [stereotaxic coordinates: -2.9 (AP), 1.3 (L) and -4.2 (DV)]. Control rats/mice were injected with an empty viral vector (AAV2/1-CMV-null, 2.48×10^{13} vg/ml) or with vehicle (sham). At different times post-injection (2, 4, 8, 16 weeks and 6, 12, 18 y 24 months) animals were subjected to behavioral tests and processed for further characterization.

Generation of transgenic rodents

Constitutive transgenic mice and rats.

Transgenic embryos were generated by microinjecting the expression cassette into zygote pronuclei that were afterwards transferred into pseudopregnant recipient females. Transgenic lines were generated using mice of a mixed genetic background (C57BL6/J and SJLF2), or rats of the Sprague Dawley strain following the same protocol. Founders were identified by Southern blotting of tail DNA using transgene specific probes. Each individual line was then backcrossed with mice or rats from the same strain and the expression level of the litters assessed by real time PCR or quantitative Western blotting. 3 lines (high, medium and low) were used to determine expression specificity in different tissues and for further characterization.

Inducible transgenic mice and rats

The expression cassettes corresponding to the tTa and the tet-O elements are microinjected in the pronuclei of Sprague Dawley rats or hybrid mice to produce two different lines of transgenic animals: (i) one *transactivator line* expressing the activator (tTA) under the control of the tissue-specific TH promoter and (ii) a *responder line* containing the tetracycline responder element (tet-O) that controls the expression of the downstream human tyrosinase gene. Conditional double-transgenics are generated by cross-breeding tet-O founder males with tTA founder females (or the other way round), and the litters backcrossed to obtain a stable line. Under normal

tetracycline or doxycycline free conditions, the double-transgenic progeny express tyrosinase with a tissue specific pattern determined by the TH promoter (including dopaminergic neurons from the *substantia nigra pars compacta* and noradrenergic neurons from the *locus coeruleus*). In the presence of doxycycline, added to food or drinking water, the system is activated resulting in the suppression of the transgene over-expression.

All founder rodents are generated at the Center of Animal Biotechnology and Gene Therapy (CBATEG) at the Universitat Autònoma de Barcelona (Barcelona, Spain). Rodents are housed in specific pathogen-free conditions, with ad libitum access to water and food, in the Animal Facility of the Vall d'Hebron Research Institute (VHIR).

All experimental procedures were approved by the Animal Experimentation Ethics Committee (35/14 CEEA) at the VHIR (Barcelona, Spain).

B) Results

Adult Sprague-Dawley rats received a single unilateral stereotaxic injection with 2 μ l of either the adeno-associated viral (AAV) vector containing the human tyrosinase gene under the control of the CMV promoter (AAV2/1-CMV-Tyr), the empty viral vector (AAV2/1-CMV-null) or the vehicle (sham) into the right substantia nigra pars compacta (SNpc) (FIG. 1a, Left panel). Delivery of AAV2/1-CMV-Tyr produced widespread expression of cytoplasmic tyrosinase in dopaminergic TH-immunoreactive neurons in ipsilateral substantia nigra (SN) and ventral tegmental area (VTA) (FIG. 1a, Right panel). Similar to humans, accumulation of NM-like brown pigment could be observed macroscopically in the injected hemisphere 4 weeks post-injection in absence of any kind of staining (FIG. 1b). No NM accumulation was observed in the contralateral (control) hemisphere, since rodents normally lack this brain pigment. Whereas tyrosinase was widely expressed throughout the injected hemisphere, accumulation of NM was restricted to the SNpc and ventral tegmental area (VTA). This accumulation pattern mimics NM distribution in human brain and indicates that NM synthesis requires enzymes or other components from dopamine metabolism. Ipsilateral SNpc pigmentation in AAV-hTyr-injected rats corresponds to the presence of intracellular NM within SNpc/VTA neurons (FIG. 1c). By 8 weeks post-AAV-hTyr injection,

intracellular NM reached levels similar to that of elder human brains, with NM occupying most of the cellular cytoplasm of SNpc neurons (FIG. 1d). As in humans, pigmented SNpc from AAV-hTyr-injected rodents can be detected by NM-sensitive high-resolution T1-weighted magnetic resonance imaging (FIG. 1e). Ultrastructurally, NM from AAV-hTyr-injected rats is indistinguishable from human NM (FIG. 1f). By 16 weeks post-AAV-hTyr injection, NM-containing SNpc neurons from AAV-hTyr-injected rats exhibit several neuropathological features typical of PD brains, such as extracellular and perivascular NM, neuronophagia, Marinesco bodies and pale bodies (FIG. 2)

To determine whether progressive NM accumulation in SNpc neurons could lead to nigro-striatal dopaminergic dysfunction/neurodegeneration, AAV-Tyr-injected rats were subjected to motor behavioral tests, such as the cylinder test, and neuropathological analyses, by stereological cell counts of dopaminergic TH-positive SNpc neurons. The cylinder test is designed to evaluate forelimb use asymmetry by quantifying the number of forelimb contacts against the wall within an open-top, clear plastic cylinder while rearing. The number of contacts with the forelimb ipsilateral or contralateral to the injection site is expressed as a percentage of total contacts.

Animals were tested both prior to AAV-Tyr injections, to exclude putative spontaneous asymmetry, and regularly every 4 weeks after AAV-Tyr inoculations. Rats were then euthanized at different time-points from 4 weeks to 16 weeks post-injection and their brains removed and processed for histochemical examination. Progressive reduction of contralateral forelimb use was observed in AAV-Tyr-injected rats starting at 4 weeks post-injection and reaching statistical significance by 8 weeks and onwards (FIG. 3, Top panel). Behavioral deficits in AAV-Tyr-injected rats were associated to progressive loss of TH-positive neurons in the ipsilateral SNpc starting at 8 weeks post-injection, compared to non-injected rats (FIG. 3, Bottom panel).

Progressive NM accumulation can also be seen in catecholaminergic brain structures, including the SNpc, VTA and LC, of 12 month-old Tg-TH-hTyr mice (FIG. 4).

As can be seen from the results discussed, it has been clearly proven that the rodents expressing the human tyrosinase gene in the SNpc not only show

remarkable neurodegeneration at the cellular level (FIG.3, Bottom panel), but also early-on motor dysfunctions typical of PD (FIG.2, Top panel).

- 5 Thus, it is concluded that the rodent of the present invention can be employed as a faithful model of the progression of PD in humans, and therefore as a promising *in vivo* screening tool for the search of novel biomarkers and therapies to control and combat the disease.

REFERENCES CITED IN THE APPLICATION

- Ikemoto, K. et.al. "Does tyrosinase exist in neuromelanin-pigmented neurons in the human substantia nigra?" *Neurosc. Lett.* 1998, vol. 11, pp. 198-200
- 5 Tief, K., et.al. "Tyrosinase, the key enzyme in melanin synthesis, is expressed in murine brain" *Eur. J. Biochem.* 1996, vol. 241, pp. 12-16
- 10 Xu, Y., et.al. "Tyrosinase mRNA is expressed in human substantia nigra" *Brain Res. Mol. Brain Res.* 1997, vol. 45, pp.159-162
- 15 Greggio E., et. al. "Tyrosinase exacerbates dopamine toxicity but is not genetically associated with Parkinson's disease" *J. of Neurochem.* 2005, vol. 93, pp. 246-256
- Olanow, W., et. al. "Parkinson's disease and alpha synuclein: Is Parkinson's disease a prion-like disorder?" *Movement Disorders* 2013, vol. 28, pp. 31-40
- 20 Blum, D., et.al. "Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease" *Prog. In Neurobiol.* 2001, vol. 65, pp. 135-172
- 25 Harvey, B.K. et. al. "Transgenic rodent models of Parkinson's disease" 2008, vol. 101, pp. 89-92
- Halliday, G.M. "Alpha-synuclein redistributes to neuromelanin lipid in the substantia nigra early in Parkinson's disease" *Brain* 2005, vol. 128, pp. 2654-2664
- 30 Hasegawa et al. "Increased dopamine and its metabolites in SH-SY5Y neuroblastoma cells that express tyrosinase" *J. Neurochem.* 2003, vol. 87, pp. 470-475
- 35 Fasano, M, et al. "Residual substantia nigra neuromelanin in Parkinson's disease is cross-linked to α -synuclein". *Neurochem Int* 2003, vol. 42, pp. 603-606

CLAIMS

1. A rodent that comprises the human tyrosinase gene, the gene being expressed in at least the dopaminergic neurons of the substantia nigra pars compacta.
5
2. A rodent according to claim 1, wherein the rodent progressively accumulates neuromelanine (NM) in said neurons along its lifetime.
- 10 3. The rodent according to claims 1-2, wherein the human tyrosinase gene is the human tyrosinase gene of SEQ ID NO 1.
4. The rodent according to any one of claims 1-3, wherein the human tyrosinase gene has been transduced with a viral vector.
15
5. The rodent according to claim 4, wherein the viral vector is an adeno-associated viral vector.
6. The rodent according to claim 5, wherein the adeno-associated viral vector
20 is the adeno-associated viral vector of SEQ ID NO 2.
7. The rodent according to any one of claims 1-3, wherein the rodent is a transgenic rodent.
- 25 8. The transgenic rodent according to claim 7, wherein the expression of the human tyrosinase gene is constitutive.
9. The transgenic rodent according to claim 8, wherein the human tyrosinase gene is operably linked to the promoter of a tyrosine hydroxylase.
30
10. The transgenic rodent according to any one of claims 8-9, departing from an embryo that has been injected with the construct comprising SEQ ID NO 3.
11. The transgenic rodent according to claim 7, wherein the expression of the
35 human tyrosinase gene is inducible.
12. The transgenic rodent according to claim 11, which is an inducible double

transgenic rodent derived from mating a first parent rodent comprising the gene coding for the tTA transactivator operably linked to the promoter of a rodent tyrosine hydroxylase, and a second parent comprising the gene coding for the human tyrosinase operably linked to a promoter under the control of the target sequence for the tTA transactivator.

13. A method of screening for therapeutic agents that prevent, delay or halt the development of neurodegenerative disease in the rodent according to any one of claims 1-12, comprising:

10 1) exposing said rodent to at least one candidate therapeutic agent; and

 2) assessing the effect of the at least one therapeutic agent on the progression of neurodegenerative disease in said rodent.

15 14. The method of screening for therapeutic agents according to claim 13, wherein the neurodegenerative disease is associated with neuromelanin accumulation.

20 15. The method of screening for therapeutic agents according to any one of claims 13-14, wherein the neurodegenerative disease is associated with α -synuclein accumulation.

25 16. A method of screening for a biomarker for the early detection, diagnosis and/or prognosis of neurodegenerative disease in the rodent according to any one of claims 1-12, comprising:

 1) isolating from said rodent and a second reference rodent at least one sample;

30 2) determining the differential expression and/or level of a potential biomarker between said rodent and the second reference rodent, wherein if there is a difference in expression and/or level of the potential biomarker, then the potential biomarker is taken as a biomarker for the early detection, diagnosis and/or prognosis of neurodegenerative disease.

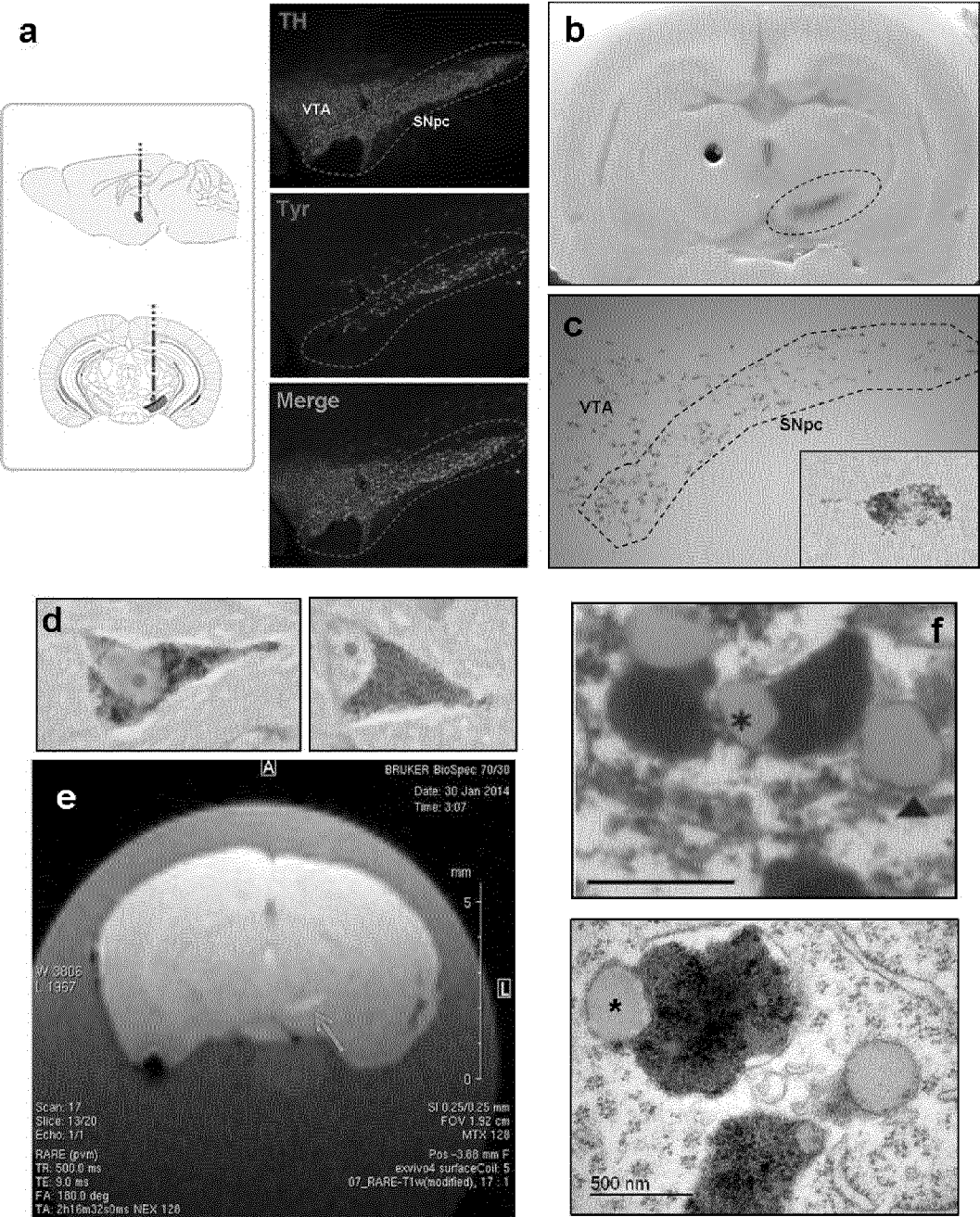


FIG.1

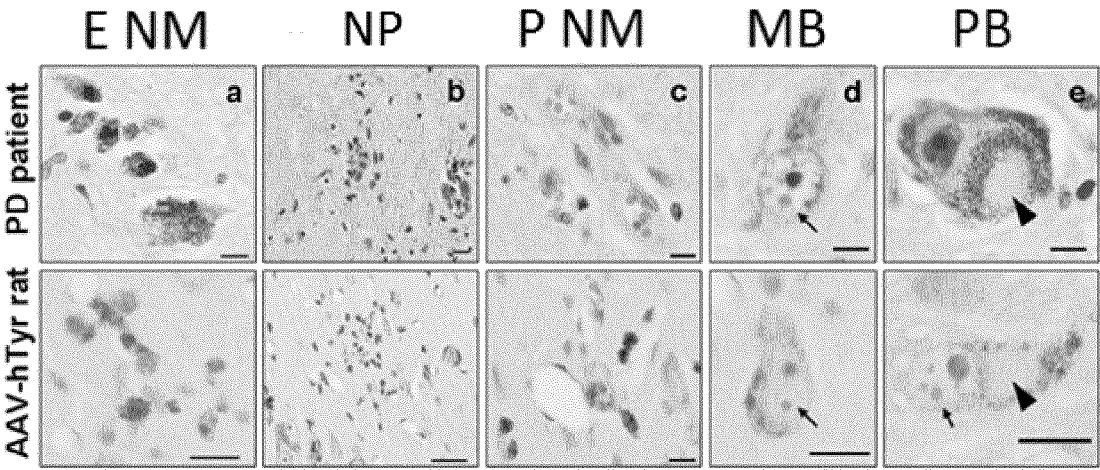


FIG.2

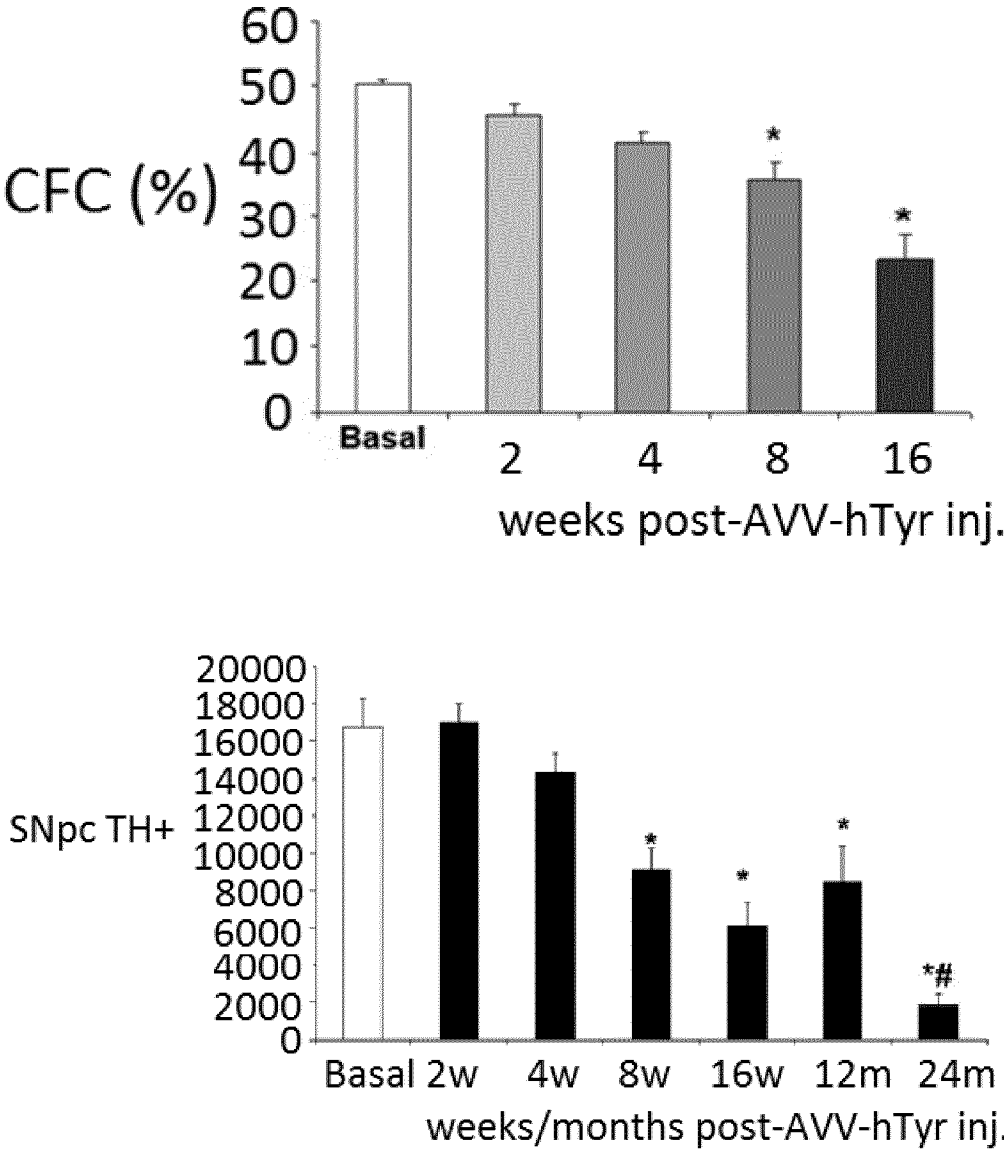


FIG.3

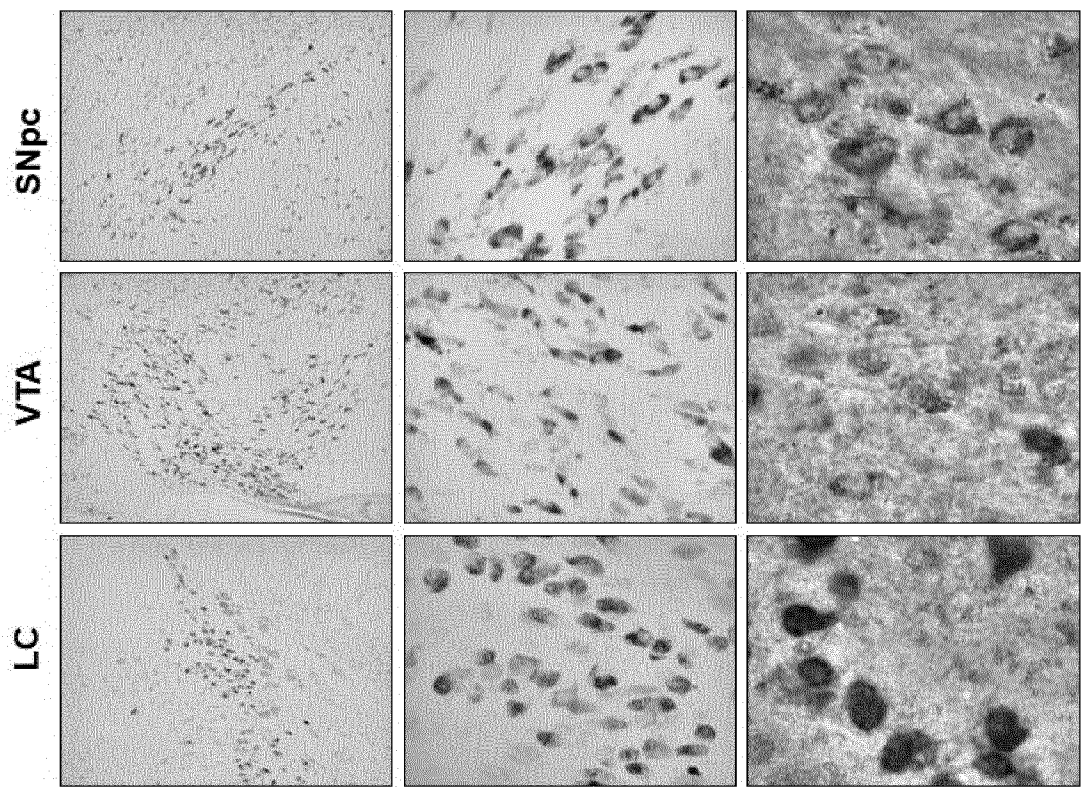


FIG.4

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/073909

A. CLASSIFICATION OF SUBJECT MATTER

INV. A01K67/027 C12N9/02 A61K49/00
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)

A01K C12N A61K

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, CHEM ABS Data, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PORTER S D ET AL: "A distal tyrosinase upstream element stimulates gene expression in neural-crest-derived melanocytes of transgenic mice: position-independent and mosaic expression", DEVELOPMENT,, vol. 120, 1994, pages 2103-2111, XP002970670, ISSN: 0950-1991	1,7,8
Y	page 2104; figures 2,3 ----- -/--	3



Further documents are listed in the continuation of Box C.



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

"E" earlier application or patent but published on or after the international filing date

"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

9 December 2015

Date of mailing of the international search report

18/12/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Deleu, Laurent

INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2015/073909

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KIRSTEN TIEF ET AL: "New evidence for presence of tyrosinase in substantia nigra, forebrain and midbrain", MOLECULAR BRAIN RESEARCH, vol. 53, no. 1-2, 1998, pages 307-310, XP055179422, ISSN: 0169-328X, DOI: 10.1016/S0169-328X(97)00301-X abstract page 3 -----	1,7,8
Y	EP 2 298 885 A1 (SYSMEX CORP [JP]) 23 March 2011 (2011-03-23) sequence 1 -----	3
A	P. MULCAHY ET AL: "Development and characterisation of a novel rat model of Parkinson's disease induced by sequential intranigral administration of AAV-[alpha]-synuclein and the pesticide, rotenone", NEUROSCIENCE, vol. 203, February 2012 (2012-02), pages 170-179, XP055179448, ISSN: 0306-4522, DOI: 10.1016/j.neuroscience.2011.12.011 page 172 - page 175 -----	2,4-6, 9-16
A	NICOLETA MOISOI ET AL: "Loss of PINK1 enhances neurodegeneration in a mouse model of Parkinson's disease triggered by mitochondrial stress", NEUROPHARMACOLOGY, vol. 77, February 2014 (2014-02), pages 350-357, XP055179573, ISSN: 0028-3908, DOI: 10.1016/j.neuropharm.2013.10.009 page 355 -----	2,4-6, 9-16
A	WO 2007/011682 A2 (UNIV CALIFORNIA [US]; SMITH MARTIN [US]; HILGENBERG LUTZ [US]) 25 January 2007 (2007-01-25) page 50, line 5 - line 10; example 10 -----	2,4-6, 9-16
A	TAKAFUMI HASEGAWA: "Tyrosinase-Expressing Neuronal Cell Line as in Vitro Model of Parkinson's Disease", INTERNATIONAL JOURNAL OF MOLECULAR SCIENCES, vol. 11, no. 3, 12 March 2010 (2010-03-12), pages 1082-1089, XP055179507, DOI: 10.3390/ijms11031082 page 1084 - page 1086 ----- -/--	2,4-6, 9-16

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/073909

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 8 502 016 B1 (JOHNSTON JENNIFER A [US]) 6 August 2013 (2013-08-06) column 10 - column 12; claims 9-17 -----	13-16
A	WO 2006/042137 A2 (MCLEAN HOSPITAL CORP [US]; ISACSON OLE [US]) 20 April 2006 (2006-04-20) page 17 - page 29 -----	13-16
A	M S OH ET AL: "Expression of transgenes in midbrain dopamine neurons using the tyrosine hydroxylase promoter", GENE THERAPY, vol. 16, no. 3, 18 September 2008 (2008-09-18), pages 437-440, XP055179512, ISSN: 0969-7128, DOI: 10.1038/gt.2008.148 abstract -----	1,9
A	ANNAGIUSI GARGIULO ET AL: "AAV-mediated Tyrosinase Gene Transfer Restores Melanogenesis and Retinal Function in a Model of Oculo-cutaneous Albinism Type I (OCA1)", MOLECULAR THERAPY, vol. 17, no. 8, 12 May 2009 (2009-05-12), pages 1347-1354, XP055179320, ISSN: 1525-0016, DOI: 10.1038/mt.2009.112 page 1351 -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2015/073909

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 2298885	A1	23-03-2011	CN 102046789 A 04-05-2011
			EP 2298885 A1 23-03-2011
			US 2011076689 A1 31-03-2011
			WO 2009145303 A1 03-12-2009
WO 2007011682	A2	25-01-2007	US 2010095387 A1 15-04-2010
			WO 2007011682 A2 25-01-2007
US 8502016	B1	06-08-2013	NONE
WO 2006042137	A2	20-04-2006	US 2006078890 A1 13-04-2006
			WO 2006042137 A2 20-04-2006