

CLAIMS

What is claimed is:

- 5 1. A genetically engineered rodent comprising in its genome a modified endogenous DR6 locus (i) comprising a deletion of the entire DR6 coding region, or a portion thereof, and (ii) comprising a nucleic acid sequence encoding a functional DR6 signal peptide operably fused to a transmembrane domain, wherein the transmembrane protein is operably fused to a reporter protein, wherein the nucleic acid sequence is operably linked to an endogenous DR6
10 transcriptional regulatory sequence, wherein any polypeptide encoded by the endogenous DR6 locus lacks a functional DR6 cytoplasmic domain, and wherein the rodent develops: (a) dysfunction of a motor neuron and/or (b) diminished nociception compared to control rodents.
- 15 2. The genetically engineered rodent of claim 1, wherein the dysfunction of a motor neuron and/or the diminished nociception compared to control rodents is not apparent at birth, and develops after 8 weeks of age.
3. The genetically modified rodent of claim 1, wherein:
20 (a) the functional DR6 signal peptide is an endogenous DR6 signal peptide;
(b) the transmembrane domain is not an endogenous DR6 transmembrane domain;
(c) the transmembrane domain is an ROR1 transmembrane domain; and/or
(d) the reporter protein is beta galactosidase.
4. The genetically modified rodent of any one of claims 1-3, wherein:
25 (a) the genetically modified rodent is heterozygous for the modified endogenous DR6 locus; and
(b) when the rodent exhibits dysfunction of a motor neuron, preferably the motor neuron is an upper motor neuron or a lower motor neuron, more preferably the dysfunction of a motor neuron is not apparent in a blinded subjective neurological scoring assay, rotarod test,
30 catwalk test, open field test, and weight measurement until the rodent is more than 4 weeks old.

5. The genetically modified rodent of any one of the preceding claims, wherein the rodent is a rat or a mouse.

6. The genetically modified rodent of claim 5, wherein the rodent is a mouse, and wherein the endogenous mouse DR6 locus comprises operably linked to at least one endogenous DR6 transcriptional regulatory sequence a nucleic acid sequence encoding an endogenous DR6 signal peptide operably fused to an ROR1 transmembrane domain that is operably fused to β -galactosidase.

7. A cell or tissue derived from a genetically modified rodent of any one of claims 1-6, preferably wherein the cell is a motor neuron or an embryonic stem cell.

8. A method of making a genetically modified rodent according to claim 1, comprising the step of replacing an endogenous nucleotide sequence encoding a full-length and endogenous mature DR6 protein with a nucleic acid sequence encoding a transmembrane domain operably linked to a reporter gene, wherein the nucleic acid is in operable linkage with an endogenous DR6 transcriptional regulatory and signal peptide sequences, and wherein any polypeptide encoded by the endogenous DR6 locus lacks a functional DR6 cytoplasmic domain.

9. A method of making a population of motor neurons that exhibits increased oxidative stress compared to control motor neuron cells, the method comprising establishing embryoid bodies from inner mass embryonic stem cells isolated from the rodent according to any one of claims 1-6 or made according to the method of claim 8, and differentiating the embryoid bodies into the population of motor neurons.

10. A method of screening a candidate agent for modulating motor neuron dysfunction comprising:

(a) administering a candidate agent to a rodent of any one of claims 1-6 or made according to the method of claim 8; and

(b) determining any modulatory effects of the candidate agent on at least one symptom of the motor neuron dysfunction in the rodent compared to a test control rodent;

wherein the presence of a modulatory effect on the at least one symptom of the motor neuron dysfunction in the rodent compared to the test control rodent is indicative that the candidate agent is useful for modulating motor neuron dysfunction.

5 11. The method of claim 10, wherein:

(a) the agent is administered prior to detection of the symptom by blinded subjective ALS-TDI neurological scoring, rotarod testing, catwalk testing, open field testing, measuring weight, or determining the latency to respond to a painful stimulus;

10 (b) the agent is administered after detection of the symptom by one or more of blinded subjective ALS-TDI neurological scoring, rotarod testing, catwalk testing, open field testing, measuring weight, or determining the latency to respond to a painful stimulus;

(c) the agent is administered at two or more different time points;

(d) the at least one symptom is selected from kyphosis, abnormal hind limb clasping, deficient motor coordination, deficient motor learning ability, weight loss, and deficient sensory
15 perception compared with a control rodent that has a strain identical to the rodent but does not comprise the nucleic acid in its genome;

(e) the at least one symptom results from an upper motor neuron dysfunction, preferably wherein the at least one symptom is selected from the group consisting of tremors, spastic paralysis (rigidity), abnormal reflexes, and a combination thereof;

20 (f) the at least one symptom results from lower motor neuron dysfunction, preferably wherein the at least one symptom is selected from the group consisting of muscle weakness and wasting, fasciculations, and a combination thereof;

(g) the at least one symptom of motor neuron dysfunction is reduced weight gain;
and/or

25 (h) the at least one symptom of motor neuron dysfunction is diminished nociception.

12. A method for screening a candidate agent for reducing oxidative stress in a motor neuron comprising:

(a) culturing motor neurons derived from a rodent according to any one of claims 1-6
30 in the presence or absence of an agent;

(b) determining whether the agent prevents, inhibits, and/or reduces oxidative stress in the motor neurons as compared to control motor neurons derived from the rodent but cultured in the absence of the agent; wherein the prevention, inhibition, and/or reduction of oxidative stress in the motor neurons is indicative of a candidate agent for reducing oxidative stress in a motor neuron.

13. A nucleic acid comprising in operably linkage and from 5' to 3', a first nucleic acid sequence encoding a DR6 signal peptide, a second nucleic acid encoding a transmembrane domain and a third nucleic acid encoding a reporter gene, and optionally a fourth nucleic acid encoding a drug selection cassette, wherein any polypeptide encoded by the nucleic acid lacks a functional DR6 cytoplasmic domain.

14. The nucleic acid sequence of claim 13:

(a) wherein the first nucleic acid sequence is set forth as SEQ ID NO: 6, the second nucleic acid sequence is set forth as SEQ ID NO: 7, the third nucleic acid sequence is set forth as SEQ ID NO: 8 and/or the fourth nucleic acid is set forth as SEQ ID NO: 3; and/or

(b) further comprising a first targeting arm and a second targeting arm, wherein the first targeting arm is 5' of and in operable linkage to the first, second and/or third nucleic acid sequences, wherein the second targeting arm is 3' of and in operable linkage to the first, second and/or third nucleic acid sequences, and wherein the first and second targeting arms are for targeting the nucleic acid for insertion in an endogenous non-human animal DR6 locus in operable linkage to at least one endogenous non-human animal regulatory element.

15. The nucleic acid of claim 14, wherein:

(a) the non-human animal is a rodent, preferably wherein the rodent is a mouse; and/or

(b) the rodent is a mouse and the nucleic acid comprises a sequence set forth as SEQ ID NO: 5 or SEQ ID NO: 17.

16. A rodent cell comprising the nucleic acid of any one of claims 13 to 15.

17. The rodent cell of claim 16, wherein the rodent cell is an embryonic stem cell.