

We claim:

1. A rodent comprising a hexanucleotide repeat expansion sequence at an endogenous C9orf72 locus, wherein the hexanucleotide repeat expansion sequence
 - (i) comprises at least one repeat of the hexanucleotide sequence set forth as SEQ ID NO: 1,
 - (ii) is in operable linkage to an endogenous C9orf72 regulatory sequence, and
 - (iii) is expressed from an endogenous C9orf72 promoter.
2. The rodent of claim 1, wherein the hexanucleotide repeat expansion sequence comprises at least thirty repeats of the hexanucleotide sequence set forth as SEQ ID NO:1.
3. The rodent of claim 1, wherein the hexanucleotide repeat expansion comprises at least 90 repeats of the hexanucleotide sequence set forth as SEQ ID NO:1.
4. The rodent of claim 1, wherein the rodent exhibits (i) increased expression of C9orf72 transcripts compared to a control rodent comprising a wildtype C9orf72 locus, (ii) an increased number of RNA foci compared to a control rodent comprising a wildtype C9orf72 locus, (iii) an increased level of dipeptide repeat proteins compared to a control rodent comprising a wildtype C9orf72 locus, or (iv) any combination of (i)-(iii).
5. The rodent of claim 1, wherein the hexanucleotide repeat expansion sequence comprises from 5' to 3': a first hexanucleotide flanking sequence, contiguous repeats of the hexanucleotide sequence set forth as SEQ ID NO: 1, and a second hexanucleotide flanking sequence,

wherein at least a portion of the non-coding sequence encompassing exon 1 and the ATG start codon of the endogenous C9orf72 locus is replaced with the hexanucleotide repeat expansion sequence.
6. The rodent of claim 5, wherein the first hexanucleotide flanking sequence comprises the sequence set forth as SEQ ID NO: 36 and the second hexanucleotide flanking sequence comprises the sequence set forth as SEQ ID NO: 37.

7. The rodent of claim 5, wherein the hexanucleotide repeat expansion sequence comprises a sequence set forth as SEQ ID NO:2, SEQ ID NO:3, or variants thereof.
8. The rodent of claim 1, wherein the rodent is a rat or a mouse.
9. The rodent of claim 8, wherein the rodent is a mouse,

wherein the endogenous C9orf72 locus comprises from 5' to 3':

(i) a mouse C9orf72 sequence comprising the sequence set forth as SEQ ID NO:20 or SEQ ID NO:23,

(ii) a heterologous repeat expansion sequence comprising a sequence set forth as SEQ ID NO:2, SEQ ID NO:3, or variants thereof,

(iii) a mouse C9orf72 sequence comprising the sequence set forth as SEQ ID NO:22 or SEQ ID NO:25, and

(iv) mouse C9orf72 coding exons, and

wherein the mouse exhibits all of the following three characteristics (i) increased expression of C9orf72 transcripts compared to a control rodent comprising a wildtype C9orf72 locus, (ii) an increased number of RNA foci compared to a control rodent comprising a wildtype C9orf72 locus, and (iii) an increased level of dipeptide repeat proteins compared to a control rodent comprising a wildtype C9orf72 locus.

10. The rodent of claim 1, wherein the rodent is homozygous for the hexanucleotide repeat expansion sequence.
11. The rodent of claim 1, wherein the rodent is heterozygous for the hexanucleotide repeat expansion sequence.

12. The rodent of claim 11, wherein the hexanucleotide repeat expansion sequence is a first hexanucleotide expansion sequence, wherein the rodent is also heterozygous for a second hexanucleotide repeat expansion sequence, and wherein the first hexanucleotide repeat expansion sequence has a different number of repeats than the second hexanucleotide repeat expansion sequence.

13. The rodent of claim 12, wherein the first hexanucleotide repeat expansion sequence comprises one to three repeats of the hexanucleotide sequence set forth as SEQ ID NO:1 and the second hexanucleotide repeat expansion sequence comprises four to one hundred repeats of the hexanucleotide sequence set forth as SEQ ID NO:1.

14. A method of identifying a therapeutic candidate for the treatment of a disease or condition associated with the presence of a hexanucleotide repeat expansion sequence, the method comprising

(a) administering a candidate agent to ~~a rodent or~~ a rodent cell comprising a hexanucleotide repeat expansion sequence at an endogenous C9orf72 locus,

wherein the hexanucleotide repeat expansion sequence

(i) comprises at least one repeat of the hexanucleotide sequence set forth as SEQ ID NO: 1,

(ii) is in operable linkage to an endogenous C9orf72 regulatory sequence, and

(iii) is expressed from an endogenous C9orf72 promoter, and

wherein the ~~rodent or~~ rodent cell exhibits (i) increased expression of C9orf72 transcripts compared to a control ~~rodent or~~ rodent cell comprising a wildtype C9orf72 locus, (ii) an increased number of RNA foci compared to a control ~~rodent or~~ rodent cell comprising a wildtype C9orf72 locus, (iii) an increased level of dipeptide repeat proteins compared to a control ~~rodent or~~ rodent cell comprising a wildtype C9orf72 locus, or (iv) any combination of (i)-(iii);

(b) performing one or more assays to measure the level of a C9orf72 gene product expressed by the ~~rodent or~~ rodent cell derived therefrom, wherein the C9orf72 gene product is selected from

the group consisting of sense C9orf72 RNA, antisense C9orf72 RNA, RNA foci comprising C9orf72 sense or antisense RNA, and dipeptide repeat proteins; and

(c) identifying the candidate agent that has an effect on the level of a C9orf72 gene product selected from the group consisting of sense C9orf72 RNA, antisense C9orf72 RNA, RNA foci comprising C9orf72 sense or antisense RNA, and dipeptide repeat proteins expressed by the rodent or rodent cell as the therapeutic candidate.

15. The method of claim 14, wherein ~~the candidate agent is administered in vivo to a rodent or rodent cell, and optionally,~~ the assay is performed in vitro ~~on tissue isolated from the rodent or rodent cell after administration of the candidate agent.~~
16. The method of claim 14, wherein the candidate agent is administered in vitro to a rodent embryonic stem cell-derived motor neuron.
17. The method of claim 14, wherein the assay is quantitative polymerase chain reaction (qPCR) to detect C9orf72 gene products.
18. The method of claim 17, wherein qPCR is performed with a primer and/or probe having a nucleotide sequence set forth in SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:80, or any combination thereof.
19. The method of claim 14, wherein the assay measures RNA foci comprising a C9orf72 sense or antisense RNA transcripts.
20. The method of claim 19, wherein the assay is fluorescent in situ hybridization using one or more probes having a nucleotide sequence as set forth in any one of SEQ ID NO:81, SEQ ID NO:82, SEQ ID NO:83, and/or SEQ ID NO:84.
21. The method of claim 14, wherein the assay measures polyGA dipeptide repeat proteins.

22. A rodent cell comprising a hexanucleotide repeat expansion sequence at an endogenous C9orf72 locus,
- wherein the hexanucleotide repeat expansion sequence
- (i) comprises at least one repeat of the hexanucleotide sequence set forth as SEQ ID NO: 1,
- (ii) is in operable linkage to an endogenous C9orf72 regulatory sequence, and
- (iii) is expressed from an endogenous C9orf72 promoter.
23. The rodent cell of claim 22, wherein the rodent cell is an embryonic stem cell, an embryonic stem cell-derived motor neuron, a brain cell, a cortical cell, a neuronal cell, a muscle cell, a heart cell, or a germ cell.
24. An immortalized cell line or motor neuron derived from the embryonic stem cell of claim 23.
25. The rodent cell of claim 22, wherein the hexanucleotide repeat expansion sequence comprises at least thirty repeats of the hexanucleotide sequence set forth as SEQ ID NO:1.
26. The rodent cell of claim 22, wherein the hexanucleotide repeat expansion sequence comprises at least 90 repeats of the hexanucleotide sequence set forth as SEQ ID NO:1.
27. The rodent cell of claim 22, wherein the rodent cell exhibits (i) increased expression of C9orf72 transcripts compared to a control rodent cell comprising a wildtype C9orf72 locus, (ii) an increased number of RNA foci compared to a control rodent cell comprising a wildtype C9orf72 locus, (iii) an increased level of dipeptide repeat proteins compared to a control rodent cell comprising a wildtype C9orf72 locus, or (iv) any combination of (i)-(iii).
28. The rodent cell of claim 22, wherein the hexanucleotide repeat expansion sequence comprises from 5' to 3': a first hexanucleotide flanking sequence, contiguous repeats of the hexanucleotide sequence set forth as SEQ ID NO: 1, and a second hexanucleotide flanking sequence,

wherein at least a portion of the non-coding sequence encompassing exon 1 and the ATG start codon of the endogenous C9orf72 locus is replaced with the hexanucleotide repeat expansion sequence.

29. The rodent cell of claim 28, wherein the first hexanucleotide flanking sequence comprises the sequence set forth as SEQ ID NO: 36 and the second hexanucleotide flanking sequence comprises the sequence set forth as SEQ ID NO: 37.

30. The rodent cell of claim 28, wherein the hexanucleotide repeat expansion sequence comprises a sequence set forth as SEQ ID NO:2, SEQ ID NO:3, or variants thereof.

31. The rodent cell of claim 22, wherein the rodent cell is a rat cell or a mouse cell.

32. The rodent cell of claim 31, wherein the rodent cell is a mouse cell,

wherein the endogenous C9orf72 locus comprises from 5' to 3':

(i) a mouse C9orf72 sequence comprising the sequence set forth as SEQ ID NO:20 or SEQ ID NO:23,

(ii) a heterologous repeat expansion sequence comprising a sequence set forth as SEQ ID NO:2, SEQ ID NO:3, or variants thereof,

(iii) a mouse C9orf72 sequence comprising the sequence set forth as SEQ ID NO:22 or SEQ ID NO:25, and

(iv) mouse C9orf72 coding exons, and

wherein the mouse cell exhibits all of the following three characteristics (i) increased expression of C9orf72 transcripts compared to a control mouse comprising a wildtype C9orf72 locus, (ii) an increased number of RNA foci compared to a control mouse comprising a wildtype C9orf72 locus, and (iii) an increased level of dipeptide repeat proteins compared to a control mouse comprising a wildtype C9orf72 locus.

33. A rodent embryonic stem cell whose genome comprises a hexanucleotide repeat expansion sequence at an endogenous C9orf72 locus,

wherein the hexanucleotide repeat expansion sequence

(i) comprises at least one repeat of the hexanucleotide sequence set forth as SEQ ID NO: 1,

(ii) is in operable linkage to an endogenous C9orf72 regulatory sequence, and

(iii) is expressed from an endogenous C9orf72 promoter.

34. A rodent embryo generated from the embryonic stem cell of claim 33.

35. A method of making a rodent whose genome comprises a hexanucleotide sequence, the method comprising

modifying the genome of the rodent so that it comprises a hexanucleotide repeat expansion sequence in an endogenous C9orf72 locus, and in operable linkage to an endogenous regulatory sequence, wherein the hexanucleotide repeat expansion sequence comprises at least one repeat of the hexanucleotide sequence set forth as SEQ ID NO: 1, and

wherein the hexanucleotide repeat expansion sequence is expressed from an endogenous C9orf72 promoter.

36. The method of claim 35, wherein the modifying is achieved by a process comprising

(a) introducing into a rodent embryonic stem cell a nucleic acid construct comprising an insert nucleic acid, wherein the insert nucleic acid comprises from 5' to 3' (i) a 5' homology arm that is homologous to a 5' target sequence of the C9orf72 locus, (ii) a hexanucleotide repeat expansion sequence, and (iii) a 3' homology arm that is homologous to a 5' target sequence of the C9orf72 locus;

(b) obtaining a genetically modified rodent embryonic stem cell from (a); and

(c) creating a rodent using the genetically modified rodent embryonic stem cell of (b).

37. The method of claim 36, wherein the insert nucleic acid further comprises between the 5' and 3' homology arms one or more genes encoding one or more selection markers.
38. The method of claim 36, wherein the insert nucleic acid further comprises one or more site-specific recombination sites between the 5' and 3' homology arms.
39. The method of claim 38, wherein the insert nucleic acid further comprises between the 5' and 3' homology arms a recombinase gene and a selection marker gene flanked by the recombinase recognition sites, which recombinase recognition sites are oriented to direct an excision.
40. The method of claim 36, wherein the 5' homology arm is identical or substantially identical to exon 1 of the endogenous C9orf72 locus, or a portion thereof.
41. The method of claim 40, wherein the 5' homology arm comprises the nucleotide sequence set forth as SEQ ID NO:20 or SEQ ID NO:23.
42. The method of claim 36, wherein the 3' homology arm is identical or substantially identical to at least a portion of intron 1 of the endogenous C9orf72 locus.
43. The method of claim 42, wherein the 3' homology arm comprises the nucleotide sequence set forth as SEQ ID NO:22 or SEQ ID NO:25.
44. The method of claim 36, wherein the insert nucleic acid comprises a nucleic acid sequence set forth in SEQ ID NO:2 or SEQ ID NO:4.
45. The method of claim 36, wherein the insert nucleic acid comprises a nucleic acid sequence set forth in SEQ ID NO:3 or SEQ ID NO:6.
46. The method of claim 36, further comprising a step of breeding the rodent generated in (c) so that a rodent homozygous for the insertion is created.
47. The method of claim 35, wherein the rodent is a rat or a mouse.

48. A rodent obtainable by the method of making a rodent whose genome comprises a hexanucleotide sequence, the method comprising modifying the genome of the rodent so that it comprises a hexanucleotide repeat expansion sequence in an endogenous C9orf72 locus, and in operable linkage to an endogenous regulatory sequence, wherein the hexanucleotide repeat expansion sequence comprises at least one repeat of the hexanucleotide sequence set forth as SEQ ID NO: 1, and wherein the hexanucleotide repeat expansion sequence is expressed from an endogenous C9orf72 promoter.