

CLAIMS

What is claimed is:

1. A non-naturally occurring polynucleotide encoding a Shank3 protein, wherein the Shank3 protein comprises an SH3 domain, a PDZ domain, a Homer binding domain, a Cortactin binding domain, and a SAM domain; wherein the SH3 domain comprises at least 90% identity to residues 474-525 of SEQ ID NO: 6 or at least 90% identity to residues 473-524 of SEQ ID NO: 5; wherein the PDZ domain comprises at least 90% identity to residues 573-662 of SEQ ID NO: 6 or at least 90% identity to residues 572-661 of SEQ ID NO: 5; wherein the Homer binding domain comprises at least 90% identity to residues 1294-1323 of SEQ ID NO: 5 or 6; wherein the Cortactin binding domain comprises at least 90% identity to residues 1400-1426 of SEQ ID NO: 5 or 6 and/or wherein the SAM domain comprises at least 90% identity to residues 1664-1729 of SEQ ID NO: 6 or at least 90% identity to residues 1663-1728 of SEQ ID NO: 5; and wherein the polynucleotide is less than 4.7 kb.
2. The polynucleotide of claim 1, wherein the polynucleotide further comprises a proline-rich region.
3. The polynucleotide of claim 1 or 2, wherein the SH3 domain comprises residues 474-525 of SEQ ID NO: 6 or residues 473-524 of SEQ ID NO: 5, the PDZ domain comprises residues 573-662 of SEQ ID NO: 6 or residues 572-661 of SEQ ID NO: 5, the Homer binding domain comprises residues 1294-1323 of SEQ ID NO: 5 or 6; the Cortactin binding domain comprises residues 1400-1426 of SEQ ID NO: 5 or 6 and/or the SAM domain comprises residues 1664-1729 of SEQ ID NO: 6 or residues 1663-1728 of SEQ ID NO: 5.
4. The polynucleotide of any one of claims 1-3, wherein the polynucleotide comprises at least 90% identity to SEQ ID NO: 1 or 2.
5. The polynucleotide of claim 4, wherein the polynucleotide comprises SEQ ID NO: 1 or 2.
6. The polynucleotide of claim 1, wherein the Shank3 protein encoded by the polynucleotide further comprises an ankyrin repeat domain.
7. The polynucleotide of claim 6, wherein the ankyrin repeat domain comprises at least 90% identity to residues 148-345 of SEQ ID NO: 6 or at least 90% identity to residues 147-313 of SEQ ID NO: 5.

8. The polynucleotide of claim 7, wherein the ankyrin repeat domain comprises residues 148-345 of SEQ ID NO: 6 or residues 147-313 of SEQ ID NO: 5.
9. The polynucleotide of any one of claims 6-8, wherein the polynucleotide comprises at least 90% identity to SEQ ID NO: 3 or 4.
10. The polynucleotide of claim 9, wherein the polynucleotide comprises SEQ ID NO: 3 or 4.
11. The polynucleotide of any one of claims 1-10, wherein the polynucleotide is less than about 4.6 kb, 4.5 kb, 4.4 kb, 4.3 kb, 4.2 kb, 4.1 kb, 4.0 kb, 3.9 kb, 3.8 kb, 3.7 kb, 3.6 kb, 3.5 kb, 3.4 kb, 3.3 kb, 3.2 kb, 3.1 kb, 3.0 kb, 2.9 kb, 2.8 kb, 2.7 kb, 2.6 kb, 2.5 kb, 2.4 kb, 2.3 kb, 2.2 kb, or 2.1 kb.
12. The polynucleotide of any of claims 1-11, wherein the Shank3 protein is less than 65% identical to SEQ ID NO: 5 or 6 over the full length of SEQ ID NO: 5 or 6.
13. The polynucleotide of any of claims 1-12, wherein the Shank3 protein comprises an amino acid sequence that is at least 90% identical to any one of SEQ ID NOs: 17-20.
14. The polynucleotide of claim 13, wherein the Shank3 protein comprises the amino acid sequence of any one of SEQ ID NOs: 17-20.
15. A Shank3 protein encoded by a polypeptide of any one of claims 1-14.
16. A vector comprising the polynucleotide of any one of claims 1-14.
17. The vector of claim 16, wherein the vector is a viral vector.
18. The vector of claim 17, wherein the vector is an AAV vector.
19. The AAV vector of claim 18, wherein the vector comprises a promoter operably linked to the polynucleotide of any one of claims 1-14.
20. The AAV vector of claim 19, wherein the polynucleotide is flanked by AAV inverted terminal repeat (ITRs).
21. The AAV vector of any one of claims 18-20, wherein the AAV vector comprises a sequence that is at least 90% identical to SEQ ID NO: 7 or 21.
22. The AAV vector of claim 21, wherein the AAV vector comprises the sequence of SEQ ID NO: 7 or 21.
23. An AAV particle comprising an AAV vector of any one of claims 19-21 and a capsid protein, wherein the capsid is of a serotype selected from AAV1, 2, 5, 6, 8, 9, rh10, and PHP.eB.
24. The AAV particle of claim 23, wherein the serotype is AAV9.
25. The AAV particle of claim 23, wherein the serotype is AAV10.
26. The AAV particle of claim 23, wherein the serotype is AAV9-PHP.eB serotype.
27. The AAV vector of claim 19, wherein the promoter is a human promoter.

28. The AAV vector of claim 27, wherein the promoter is hSyn1.
29. A method comprising administering the AAV vector of any one of claims 18-21 or the AAV particle of claims 23-26 to a subject in need thereof.
30. The method of claim 29, wherein the AAV vector or particle is administered intravenously.
31. The method of claim 29, wherein the AAV vector or particle is delivered to the brain of the subject.
32. The method of claim 29, wherein the AAV vector or particle is delivered to the cortex, striatum and/or thalamus of the subject.
33. The method of claim 29, wherein the subject is a human subject.
34. The method of claim 33, wherein the human subject is an adult.
35. The method of claim 33, wherein the human subject is not an adult.
36. The method of claim 33, wherein the human subject is not older than 25 years old.
37. The method of claim 35, wherein the human subject is 10 years old or younger.
38. The method of any one of claims 28-36, wherein the subject has, is suspected of having, or is at risk of having, a neurodevelopmental disorder.
39. The method of any one of claims 29-37, wherein the subject has, is suspected of having, or is at risk of having, an autism spectrum disorder (ASD).
40. The method of any one of claims 29-37, wherein the subject exhibits one or more symptoms of an ASD.
41. The method of any one of claims 29-37, wherein the subject has, is suspected of having, or is at risk of having, Phelan–McDermid syndrome.
42. The method of any one of claims 29-41, wherein the subject exhibits one or more of: developmental delay, intellectual disability (ID), sleep disturbance, hypotonia, lack of speech, or language delay.
43. The method of any one of claims 29-42, wherein the subject has, is suspected of having, or is at risk of having, reduced expression of the Shank3 gene relative to a control subject.
44. The method of claim 43, wherein the control subject is a subject that does not have, is not suspected of having, or is not at risk of having, a neurodevelopmental disorder, an autism spectrum disorder (ASD), and/or Phelan–McDermid syndrome.
45. The method of claim 43 or 44, wherein reduced expression of the Shank3 gene is caused by disruption of at least one copy of the Shank3 gene.
46. The method of claim 45, wherein disruption of the Shank3 gene comprises a deletion in at least one copy of the Shank3 gene.

47. The method of claim 45, wherein disruption of the Shank3 gene comprises one or more mutations within at least one copy of the Shank3 gene.
48. A method of treating a subject having a neurodevelopmental disorder, the method comprising administering to the subject an effective amount of a composition comprising an AAV vector, wherein the AAV vector comprises a polynucleotide encoding a Shank3 protein.
49. A method of treating a subject having an autism spectrum disorder (ASD), the method comprising administering to the subject an effective amount of a composition comprising an AAV vector, wherein the AAV vector comprises a polynucleotide encoding a Shank3 protein.
50. A method of treating a subject having Phelan–McDermid syndrome, the method comprising administering to the subject an effective amount of a composition comprising an AAV vector, wherein the AAV vector comprises a polynucleotide encoding a Shank3 protein.
51. The method of any one of claims 48-50, wherein the Shank3 protein comprises an SH3 domain, a PDZ domain, a Homer binding domain, a Cortactin binding domain, and a SAM domain.
52. The method of any one of claims 48-50, wherein the Shank3 protein further comprises an ankyrin repeat domain.
53. The method of claim 52, wherein the ankyrin repeat domain comprises at least 90% identity to residues 148-345 of SEQ ID NO: 6 or at least 90% identity to residues 147-313 of SEQ ID NO: 5.
54. The method of any one of claims 51-53, wherein the SH3 domain comprises at least 90% identity to residues 474-525 of SEQ ID NO: 6 or at least 90% identity to residues 473-524 of SEQ ID NO: 5;
- wherein the PDZ domain comprises at least 90% identity to residues 573-662 of SEQ ID NO: 6 or at least 90% identity to residues 572-661 of SEQ ID NO: 5;
- wherein the Homer binding domain comprises at least 90% identity to residues 1294-1323 of SEQ ID NO: 5 or 6;
- wherein the Cortactin binding domain comprises at least 90% identity to residues 1400-1426 of SEQ ID NO: 5 or 6; and/or
- wherein the SAM domain comprises at least 90% identity to residues 1664-1729 of SEQ ID NO: 6 or at least 90% identity to residues 1663-1728 of SEQ ID NO: 5.

55. The method of any one of claims 48-50, wherein the SH3 domain comprises residues 474-525 of SEQ ID NO: 6 or residues 473-524 of SEQ ID NO: 5, wherein the PDZ domain comprises residues 573-662 of SEQ ID NO: 6 or residues 572-661 of SEQ ID NO: 5, wherein the Homer binding domain comprises residues 1294-1323 of SEQ ID NO: 5 or 6; wherein the Cortactin binding domain comprises residues 1400-1426 of SEQ ID NO: 5 or 6; and/or wherein the SAM domain comprises residues 1664-1729 of SEQ ID NO: 6 or residues 1663-1728 of SEQ ID NO: 5.
56. The method of any one of claims 47-49, wherein the polynucleotide is less than 4.7 kb.
57. The method of any one of claims 48-50, wherein the polynucleotide is less than about 4.6 kb, 4.5 kb, 4.4 kb, 4.3 kb, 4.2 kb, 4.1 kb, 4.0 kb, 3.9 kb, 3.8 kb, 3.7 kb, 3.6 kb, 3.5 kb, 3.4 kb, 3.3 kb, 3.2 kb, 3.1 kb, 3.0 kb, 2.9 kb, 2.8 kb, 2.7 kb, 2.6 kb, 2.5 kb, 2.4 kb, 2.3 kb, 2.2 kb, or 2.1 kb.
58. The method of any one of claims 48-50, wherein the polynucleotide further comprises a proline-rich region.
59. The method of any one of claims 48-50, wherein the polynucleotide comprises at least 90% identity to any one of SEQ ID NOs: 1-4.
60. The method of any one of claims 48-50, wherein the polynucleotide comprises any one of SEQ ID NOs: 1-4.
61. The method of any one of claims 47-50, wherein the subject is a human subject.
62. The method of claim 61, wherein the human subject is an adult.
63. The method of claim 61, wherein the human subject is not an adult.
64. The method of claim 62, wherein the human subject is not older than 25 years old.
65. The method of claim 63, wherein the human subject is 10 years old or younger.
66. The method of any one of claims 48-65, wherein the composition is administered intravenously.
67. The method of any one of claims 48-65, wherein the composition is delivered to the brain of the subject.
68. The method of any one of claims 48-65, wherein the composition is delivered to the striatum and/or thalamus of the subject.
69. The method of any one of claims 48-68, wherein the subject exhibits one or more of: developmental delay, intellectual disability (ID), sleep disturbance, hypotonia, lack of speech, or language delay.

70. The method of claim 48, wherein the autism spectrum disorder (ASD) comprises autism disorder.
71. The method of any one of claims 48-70, wherein the subject has, is suspected of having, or is at risk of having, reduced expression of the Shank3 gene relative to a control subject.
72. The method of claim 71, wherein the control subject is a subject that does not have, is not suspected of having, or is not at risk of having, a neurodevelopmental disorder, an autism spectrum disorder (ASD), and/or Phelan–McDermid syndrome.
73. The method of claim 71 or 72, wherein reduced expression of the Shank3 gene is caused by disruption of at least one copy of the Shank3 gene.
74. The method of claim 73, wherein disruption of the Shank3 gene comprises a deletion in at least one copy of the Shank3 gene.
75. The method of claim 73, wherein disruption of the Shank3 gene comprises one or more mutations within at least one copy of the Shank3 gene.
76. The method of any one of claims 48-50, wherein the subject has improved sleep efficiency after being administered to an effective amount of the composition.
77. The method of any one of claims 48-76, wherein the composition is in a pharmaceutically acceptable carrier.
78. A MiniShank3 protein comprising a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to any one of SEQ ID NOs: 17-20.
79. The MiniShank3 protein of claim 78 wherein the MiniShank3 protein comprises the sequence of any one of SEQ ID NOs: 17-20.

Claims

1. A non-naturally occurring polynucleotide encoding a Shank3 protein,
 wherein the Shank3 protein comprises an SH3 domain, a PDZ domain, a Homer binding domain, a Cortactin binding domain, and a SAM domain;
 wherein the SH3 domain comprises at least 90% identity to residues 474-525 of SEQ ID NO: 6 or at least 90% identity to residues 473-524 of SEQ ID NO: 5;
 wherein the PDZ domain comprises at least 90% identity to residues 573-662 of SEQ ID NO: 6 or at least 90% identity to residues 572-661 of SEQ ID NO: 5;
 wherein the Homer binding domain comprises at least 90% identity to residues 1294-1323 of SEQ ID NO: 5 or 6;
 wherein the Cortactin binding domain comprises at least 90% identity to residues 1400-1426 of SEQ ID NO: 5 or 6; and/or
 wherein the SAM domain comprises at least 90% identity to residues 1664-1729 of SEQ ID NO: 6 or at least 90% identity to residues 1663-1728 of SEQ ID NO: 5; and
 wherein the polynucleotide is less than 4.7 kb.

2. The polynucleotide of claim 1, wherein the polynucleotide further comprises a proline-rich region, and/or wherein:
 - (i) the SH3 domain comprises residues 474-525 of SEQ ID NO: 6 or residues 473-524 of SEQ ID NO: 5;
 - (ii) the PDZ domain comprises residues 573-662 of SEQ ID NO: 6 or residues 572-661 of SEQ ID NO: 5;
 - (iii) the Homer binding domain comprises residues 1294-1323 of SEQ ID NO: 5 or 6;
 - (iv) the Cortactin binding domain comprises residues 1400-1426 of SEQ ID NO: 5 or 6;
 - (v) the SAM domain comprises residues 1664-1729 of SEQ ID NO: 6 or residues 1663-1728 of SEQ ID NO: 5;
 - (vi) the polynucleotide comprises at least 90% identity to SEQ ID NO: 1 or 2 or comprises SEQ ID NO: 1 or 2; and/or

(vii) the Shank3 protein encoded by the polynucleotide further comprises an ankyrin repeat domain, wherein the ankyrin repeat domain comprises at least 90% identity to residues 148-345 of SEQ ID NO: 6 or at least 90% identity to residues 147-313 of SEQ ID NO: 5 or comprises residues 148-345 of SEQ ID NO: 6 or residues 147-313 of SEQ ID NO: 5.

3. The polynucleotide of claim 1 or 2, wherein the polynucleotide comprises at least 90% identity to SEQ ID NO: 3 or 4 or comprises SEQ ID NO: 3 or 4.

4. The polynucleotide of any of claims 1-3, wherein the Shank3 protein encoded by the polynucleotide comprises an amino acid sequence that is at least 90% identical to any one of SEQ ID NOs: 17-20 or comprises the amino acid sequence of any one of SEQ ID NOs: 17-20.

5. A Shank3 protein encoded by the polynucleotide of any one of claims 1-4.

6. A vector comprising the polynucleotide of any one of claims 1-4, wherein the vector is a viral vector or an AAV vector.

7. The vector of claim 6, wherein the AAV vector comprises a promoter operably linked to the polynucleotide of any one of claims 1-4, wherein the promoter is a human promoter, optionally wherein the promoter is hSyn1, and/or wherein the polynucleotide is flanked by AAV inverted terminal repeat (ITRs), and/or wherein the AAV vector comprises a sequence that is at least 90% identical to SEQ ID NO: 7 or 21 or comprises the sequence of SEQ ID NO: 7 or 21.

8. An AAV particle comprising the vector of claim 6 or 7, wherein the vector is an AAV vector, and a capsid protein, and wherein the capsid is of a serotype selected from AAV1, 2, 5, 6, 8, 9, rh10, and PHP.eB.

9. Use of the vector of claim 6 or 7, wherein the vector is an AAV vector, or the AAV particle of claim 8 in the manufacture of a medicament for treating a subject having, is suspected of having, or is at risk of having, a neurodevelopmental disorder, an autism spectrum disorder (ASD), or Phelan–McDermid syndrome and/or wherein the subject exhibits one or more

symptoms of an ASD and/or wherein the subject exhibits one or more of: developmental delay, intellectual disability (ID), sleep disturbance, hypotonia, lack of speech, or language delay, wherein the AAV vector or the AAV particle is to be administered to the subject in need thereof.

10. The use of claim 9, wherein the subject has, is suspected of having, or is at risk of having, reduced expression of the Shank3 gene relative to a control subject, wherein reduced expression of the Shank3 gene is caused by disruption of at least one copy of the Shank3 gene, optionally wherein disruption of the Shank3 gene comprises a deletion in at least one copy of the Shank3 gene or one or more mutations within at least one copy of the Shank3 gene.

11. Use of a composition comprising an AAV vector, wherein the AAV vector comprises a polynucleotide encoding a Shank3 protein, in the manufacture of a medicament for treating neurodevelopmental disorder, autism spectrum disorder (ASD), or Phelan–McDermid syndrome in a subject.

12. The use of claim 11, wherein the Shank3 protein comprises an SH3 domain, a PDZ domain, a Homer binding domain, a Cortactin binding domain, and a SAM domain, and wherein:

(i) the Shank3 protein further comprises an ankyrin repeat domain, optionally wherein the ankyrin repeat domain comprises at least 90% identity to residues 148-345 of SEQ ID NO: 6 or at least 90% identity to residues 147-313 of SEQ ID NO: 5;

(ii) the SH3 domain comprises at least 90% identity to residues 474-525 of SEQ ID NO: 6 or at least 90% identity to residues 473-524 of SEQ ID NO: 5;

(iii) the PDZ domain comprises at least 90% identity to residues 573-662 of SEQ ID NO: 6 or at least 90% identity to residues 572-661 of SEQ ID NO: 5;

(iv) the Homer binding domain comprises at least 90% identity to residues 1294-1323 of SEQ ID NO: 5 or 6;

(v) the Cortactin binding domain comprises at least 90% identity to residues 1400-1426 of SEQ ID NO: 5 or 6;

(vi) the SAM domain comprises at least 90% identity to residues 1664-1729 of SEQ ID NO: 6 or at least 90% identity to residues 1663-1728 of SEQ ID NO: 5;

(vii) the SH3 domain comprises residues 474-525 of SEQ ID NO: 6 or residues 473-524 of SEQ ID NO: 5;

(viii) the PDZ domain comprises residues 573-662 of SEQ ID NO: 6 or residues 572-661 of SEQ ID NO: 5;

(ix) the Homer binding domain comprises residues 1294-1323 of SEQ ID NO: 5 or 6;

(x) the Cortactin binding domain comprises residues 1400-1426 of SEQ ID NO: 5 or 6;
and/or

(xi) the SAM domain comprises residues 1664-1729 of SEQ ID NO: 6 or residues 1663-1728 of SEQ ID NO: 5.

13. The use of claim 11 or 12, wherein the polynucleotide is less than 4.7 kb or less than about 4.6 kb, 4.5 kb, 4.4 kb, 4.3 kb, 4.2 kb, 4.1 kb, 4.0 kb, 3.9 kb, 3.8 kb, 3.7 kb, 3.6 kb, 3.5 kb, 3.4 kb, 3.3 kb, 3.2 kb, 3.1 kb, 3.0 kb, 2.9 kb, 2.8 kb, 2.7 kb, 2.6 kb, 2.5 kb, 2.4 kb, 2.3 kb, 2.2 kb, or 2.1 kb.

14. The use of any of claims 11-13, wherein the polynucleotide further comprises a proline-rich region, optionally wherein the polynucleotide comprises at least 90% identity to any one of SEQ ID NOs: 1-4 or comprises any one of SEQ ID NOs: 1-4.

15. The use of any one of claims 11-14, wherein the subject is a human subject.

16. The use of claim 15, wherein the human subject is an adult.

17. The use of claim 15, wherein the human subject is not older than 25 years old, optionally wherein the human subject is 10 years old or younger.

18. The use of any one of claims 11-17, wherein the composition is to be intravenously administered and/or delivered to the brain, striatum and/or thalamus of the subject.

19. The use of any one of claims 11-18, wherein the composition is in a pharmaceutically acceptable carrier.

20. A MiniShank3 protein comprising a sequence that is at least 80%, at least 85%, at least 90%, or at least 95% identical to any one of SEQ ID NOs: 17-20, or comprises the sequence of any one of SEQ ID NOs: 17-20.
21. The polynucleotide of any one of claims 1-4, or the shank protein of claim 5, or the vector of claim 6 or 7, or the AAV particle of claim 8 for use as a medicament.
22. A composition comprising an AAV vector, wherein the AAV vector comprises a polynucleotide encoding a Shank3 protein for use as a medicament.