[0006] The aim of medical treatment of WD is to remove the toxic deposit of copper from the body and to prevent its reaccumulation. Current treatment approaches for WD are daily oral therapy with chelating agents (D-penicillamine, trientine, and zinc salts). Medical therapy is effective in most, but not all WD patients. Liver transplantation is a therapeutic option in WD patients presenting with fulminant liver failure or progressive liver failure. However, transplant recipients are required to maintain a constant immune suppression regimen to prevent rejection.

[0007] The present invention addresses the need for improved and sustainable treatment of WD by delivering a gene expressing truncated yet functional ATP7B to patients with an adeno-associated viral vector. The truncated ATP7B of the present invention has improved efficacy in treating WD and possesses an advantage of manufacturing ease and efficiency over wild-type and other truncated forms of ATP7B protein.

SUMMARY

[0008] This present disclosure provides compositions and methods of their use in gene therapy. Provided herein are adeno-associated virus (AAV) vectors useful for the treatment of WD. In one example, the present disclosure provides a recombinant nucleic acid construct comprising: a 5'-inverted terminal repeat (ITR) sequence; a promoter sequence; a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) in which metal-binding domains (MBDs) 1-3 have been deleted, but the serine-rich loop including two serine residues (S340 and S341) between MBD3 and MBD4 is present; and a 3'-ITR sequence.

[0008a] In one aspect of the present disclosure, there is provided a recombinant nucleic acid construct comprising:

(a) a 5'-inverted terminal repeat (ITR) sequence;

(b) a promoter sequence;

(c) a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) in which metal-binding domains (MBDs) 1-3 have been deleted, but the serine-rich loop including two serine residues (S340 and S341) between MBD3 and MBD4 is present; and

a 3'-ITR sequence.

[0008b] In another aspect of the present disclosure, there is provided a use of a recombinant adeno-associated virus (rAAV) comprising an AAV capsid and a vector genome packaged therein in the manufacture of a medicament for the treatment of Wilson disease, said vector genome comprising:

a. an AAV 5'-inverted terminal repeat (ITR) sequence;

b. a promoter/enhancer sequence;

c. a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) in which metal-binding domains (MBDs) 1-3 have been deleted, but the serine-rich loop including two serine residues (S340 and S341) between MBD3 and MBD4 is present; and

d. an AAV 3'-ITR.

[0008c] In a further aspect of the present disclosure, there is provided a recombinant nucleic acid construct comprising:

a. an AAV 5'-inverted terminal repeat (ITR) sequence of SEQ ID NO:2;

b. an enhancer sequence of SEQ ID NO:3;

c. a promoter sequence of SEQ ID NO:12;

d. a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) of SEQ ID NO:1 or SEQ ID NO:15; and

e. an AAV 3'-ITR of SEQ ID NO:2.

[0008d] In another aspect of the present disclosure, there is provided a rAAV comprising an AAV capsid and a vector genome packaged therein, said vector genome comprising:

a. an AAV 5'-inverted terminal repeat (ITR) sequence of SEQ ID NO:2;

b. an enhancer sequence of SEQ ID NO:3;

c. a promoter sequence of SEQ ID NO:12;

d. a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) of SEQ ID NO:1 or SEQ ID NO:15; and

e. an AAV 3'-ITR of SEQ ID NO:2.

[0008e] In a further aspect of the present disclosure, there is provided a host cell comprising a recombinant nucleic acid construct disclosed herein.

[0008f] In another aspect of the present disclosure, there is provided a composition comprising the rAAV disclosed herein, and a pharmaceutically acceptable carrier.

[0008g] In a further aspect of the present disclosure, there is provided a recombinant vector comprising the recombinant nucleic acid construct disclosed herein.

[0008h] In another aspect of the present disclosure, there is provided a method of increasing rAAV yield for use in the treatment of Wilson disease, comprising delivering the recombinant vector disclosed herein, a packaging plasmid, and a helper plasmid to a eukaryotic host cell culture and harvesting the rAAV from the eukaryotic host cell culture.

[0009] **[0008i]** In a further aspect of the present disclosure, there is provided a recombinant nucleic acid comprising a nucleic acid sequence which is at least 80% identical to SEQ ID NO:14.In another example, the present disclosure provides a recombinant adeno-associated virus (rAAV) useful for the treatment of Wilson disease, in which rAAV comprises an AAV capsid and a vector genome packaged therein, the vector genome comprises a 5'-inverted terminal repeat (ITR) sequence; a promoter sequence; a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) in which metal-binding domains (MBDs) 1-3 have been deleted, but the serine-rich loop including two serine residues (S340 and S341) between MBD3 and MBD4 is present; and a 3'-ITR sequence.

[0010] These and other examples and features of the present disclosure are described in the following sections of the application.

WHAT IS CLAIMED IS:

1. A recombinant nucleic acid construct comprising:

(a) a 5'-inverted terminal repeat (ITR) sequence;

(b) a promoter sequence;

(c) a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) in which metal-binding domains (MBDs) 1-3 have been deleted, but the serine-rich loop including two serine residues (S340 and S341) between MBD3 and MBD4 is present; and

(d) a 3'-ITR sequence.

2. The recombinant nucleic acid construct according to claim 1, wherein the promoter sequence is selected from a transthyretin (TTR) promoter, a chicken β-actin (CBA) promoter, a cytomegalovirus (CMV) immediate early gene promoter, a thyroxine binding globulin (TBG) promoter, an alpha-1 anti-trypsin (A1AT) promoter, and a CAG promoter.

3. The recombinant nucleic acid construct according to claim 1 or 2, wherein the 5'-ITR sequence and/or the 3'-ITR sequence are from AAV2.

4. The recombinant nucleic acid construct according to any of claims 1-3, wherein the 5'-ITR sequence and the 3'-ITR sequence comprises or consists of SEQ ID NO:2.

5. The recombinant nucleic acid construct according to claim 1 or 2, wherein the 5'-ITR sequence and/or the 3'-ITR sequence are from a non-AAV2 source.

6. The recombinant nucleic acid construct according to any of claims 1-5, wherein the recombinant nucleic acid construct further comprises one or more enhancer sequences.

7. The recombinant nucleic acid construct according to claim 6, wherein the enhancer sequence is selected from a transthyretin enhancer (enTTR), a cytomegalovirus immediate early (CMV) gene enhancer, a chicken β-actin (CBA) enhancer, an En34 enhancer, and an apolipoprotein (ApoE) enhancer.

8. The recombinant nucleic acid construct according to claim 7, wherein the enhancer sequence comprises or consists of SEQ ID NO:3.

9. The recombinant nucleic acid construct according to claims 7-8, wherein the enhancer sequence is located upstream of the promoter sequence.

10. The recombinant nucleic acid construct according to any of claims 1-9, wherein the recombinant nucleic acid construct further comprises one or more intron sequences.

11. The recombinant nucleic acid construct according to claim 10, wherein the intron sequence is selected from an SV40 Small T intron, a rabbit hemoglobin subunit beta (rHBB) intron, a human beta globin IVS2 intron, a Promega chimeric intron, and an hFIX intron.

12. The recombinant nucleic acid construct according to claim 11, wherein the intron sequence comprises or consists of SEQ ID NO:4 or SEQ ID NO: 5.

13. The recombinant nucleic acid construct according to any of claims 1-12, wherein the recombinant nucleic acid construct further comprises a polyadenylation signal sequence.

14. The recombinant nucleic acid construct according to claim 13, wherein the polyadenylation signal sequence is selected from an SV40 polyadenylation signal sequence, a bovine growth hormone (BGH) polyadenylation signal sequence, and a rabbit beta globin polyadenylation signal sequence.

15. The recombinant nucleic acid construct according to claim 14, wherein the polyadenylation signal sequence comprises or consists of SEQ ID NO:6 or SEQ ID NO: 7.

16. Use of a recombinant adeno-associated virus (rAAV) comprising an AAV capsid and a vector genome packaged therein in the manufacture of a medicament for the treatment of Wilson disease, said vector genome comprising:

a. an AAV 5'-inverted terminal repeat (ITR) sequence;

b. a promoter/enhancer sequence;

c. a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) in which metal-binding domains (MBDs) 1-3 have been deleted, but the

serine-rich loop including two serine residues (S340 and S341) between MBD3 and MBD4 is present; and

d. an AAV 3'-ITR.

17. The use according to claim 16, wherein the AAV capsid is from an AAV of serotype 9, 8, 1, 2, 3, 4, 5, 6, 7, 10, 11, 12, rh10, or hu37.

18. The use according to claim 17, wherein the AAV capsid is from AAV9.

19. The use according to claim 17, wherein the AAV capsid is from AAV8.

20. The use according to claim 18, wherein the AAV capsid is an AAV9 variant capsid.

21. The use according to any of claims 16-20, wherein the promoter sequence is selected from a transthyretin (TTR) promoter, a chicken β-actin (CBA) promoter, a cytomegalovirus (CMV) immediate early gene promoter, a thyroxine binding globulin (TBG) promoter, an alpha-1 anti-trypsin (A1AT) promoter, and a CAG promoter.

22. The use according to any of claims 16-21, wherein the 5'-ITR sequence and/or the 3'-ITR sequence are from AAV2.

23. The use according to any of claims 16-22, wherein the 5'-ITR sequence and the 3'-ITR sequence comprises or consists of SEQ ID NO:2.

24. The use according to any of claims 16-21, wherein the 5'-ITR sequence and/or the 3'-ITR sequence are from a non-AAV2 source.

25. The use according to any of claims 16-24, wherein the packaged vector genome further comprises one or more enhancer sequences.

26. The use according to claim 25, wherein the enhancer sequence is selected from a transthyretin enhancer (enTTR), a cytomegalovirus (CMV) immediate early geneenhancer, a chicken β-actin (CBA) enhancer, an En34 enhancer, and an apolipoprotein (ApoE) enhancer.

27. The use according to claim 26, wherein the enhancer sequence comprises or consists of SEQ ID NO:3.

28. The use according to claim 27, wherein the enhancer sequence is located upstream of the promoter sequence.

29. The use according to any of claims 16-28, wherein the packaged vector genome further comprises one or more intron sequences.

30. The use according to claim 29, wherein the intron sequence is selected from an SV40 Small T intron, a rabbit hemoglobin subunit beta (rHBB) intron, a human beta globin IVS2 intron, a Promega chimeric intron, and an hFIX intron.

31. The use according to claim 30, wherein the intron sequence comprises or consists of SEQ ID NO:4 or SEQ ID NO: 5.

32. The use according to any of claims 16-31, wherein the packaged vector genome further comprises a polyadenylation signal sequence.

33. The use according to claim 32, wherein the polyadenylation signal sequence is selected from an SV40 polyadenylation signal sequence, a bovine growth hormone (BGH) polyadenylation signal sequence, and a rabbit beta globin polyadenylation signal sequence.

34. The use according to claim 33, wherein the polyadenylation signal sequence comprises or consists of SEQ ID NO:6 or SEQ ID NO: 7.

35. A recombinant nucleic acid construct comprising:

a. an AAV 5'-inverted terminal repeat (ITR) sequence of SEQ ID NO:2;

b. an enhancer sequence of SEQ ID NO:3;

c. a promoter sequence of SEQ ID NO:12;

d. a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) of SEQ ID NO:1 or SEQ ID NO:15; and

e. an AAV 3'-ITR of SEQ ID NO:2.

36. The recombinant nucleic acid construct of claim 35, wherein the nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) is SEQ ID NO:1.

37. The recombinant nucleic acid construct of claim 35, wherein the nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) is SEQ ID NO:15.

38. A rAAV comprising an AAV capsid and a vector genome packaged therein, said vector genome comprising:

a. an AAV 5'-inverted terminal repeat (ITR) sequence of SEQ ID NO:2;

b. an enhancer sequence of SEQ ID NO:3;

c. a promoter sequence of SEQ ID NO:12;

d. a nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) of SEQ ID NO:1 or SEQ ID NO:15; and

e. an AAV 3'-ITR of SEQ ID NO:2.

39. The rAAV of claim 38, wherein the vector genome comprises the nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) of SEQ ID NO:1.

40. The rAAV of claim 38, wherein the vector genome comprises the nucleic acid sequence encoding a truncated human copper-transporting ATPase 2 (ATP7B) of SEQ ID NO:15.

41. A host cell comprising a recombinant nucleic acid construct according to any of claims 1-15 or 35-37.

42. A composition comprising the rAAV of any of claims 16-34 or 38-40, and a pharmaceutically acceptable carrier.

43. A recombinant vector comprising the recombinant nucleic acid construct of any one of claims 1-15 or 35-37.

44. A method of increasing rAAV yield for use in the treatment of Wilson disease, comprising delivering the recombinant vector of claim 43, a packaging plasmid, and a helper plasmid to a eukaryotic host cell culture and harvesting the rAAV from the eukaryotic host cell culture.

45. A recombinant nucleic acid comprising a nucleic acid sequence which is at least 80% identical to SEQ ID NO:14.

46. The recombinant nucleic acid of claim 45, wherein the nucleic acid sequence comprises SEQ ID NO:14.