

There are significant challenges to overcome in developing a successful gene therapy for AS. The first is that the COL4A5, COL4A3 and COL4A4 proteins are 1685, 1670 and 1690 amino acids each, rendering them challenging for transport by an adeno-associated virus (AAV) vector, due to limited AAV cargo capacity. The second significant challenge is to successfully deliver the gene therapy to podocyte cells in the glomerulus of the kidney, which produce collagen IV in the glomerular basement membrane.

The present invention aims to provide a novel gene therapy vector that can efficiently deliver a COL4A3, COL4A4 or COL4A5 transgene to podocytes and thereby provide a therapy for the treatment of Alport syndrome.

SUMMARY OF THE INVENTION

The present ~~invention~~disclosure provides a viral vector, wherein the viral vector comprises a COL4A3, COL4A4 or COL4A5 transgene. The viral vector can be used to target podocytes within the glomerulus of the kidney in order to treat Alport syndrome.

Without being bound by theory, the present inventors believe that podocytes offer a highly tractable target for gene therapy approaches in kidney disease and that by targeting COL4A3, COL4A4 or COL4A5 to podocytes the collagen IV α 345 network of the glomerular basement membrane can be changed and at least partially normalised.

In one aspect, present ~~invention~~disclosure provides a viral vector, wherein the viral vector comprises a COL4A3, COL4A4 or COL4A5 transgene.

The COL4A3 transgene may encode a COL4A3 polypeptide which comprises or consists of the polypeptide sequence having at least 70% identity to SEQ ID NO: 1, or a fragment thereof; the COL4A4 transgene may encode a COL4A4 polypeptide which comprises or consists of the polypeptide sequence having at least 70% identity to SEQ ID NO: 2, or a fragment thereof; and/or the COL4A5 transgene may encode a COL4A5 polypeptide which comprises or consists of the polypeptide sequence having at least 70% identity to SEQ ID NO: 3, or a fragment thereof. In some ~~embodiments~~examples, the COL4A3 transgene encodes a full-length COL4A3 polypeptide, the COL4A4 transgene encodes a full-length COL4A4 polypeptide; and/or the COL4A5 transgene encodes a full-length COL4A5

polypeptide. Suitably, the COL4A3, COL4A4 or COL4A5 transgene is human and/or comprises a hemagglutinin (HA) tag.

Preferably, the viral vector comprises a podocyte-specific promoter. Suitably, the podocyte-specific promoter is minimal nephrin promoter NPHS1 or podocin promoter NPHS2. In some embodimentsexamples, the podocyte-specific promoter is minimal nephrin promoter NPHS1.

The present inventors have developed a minimal nephrin promoter which is shorter than known minimal nephrin promoters and surprisingly capable of driving transgene expression in podocytes. The promoter also surprisingly retains podocyte-specificity. Such a minimal nephrin promoter can be used to minimise the cargo size and aid packaging of full length COL4A3, COL4A4 or COL4A5. Accordingly, the minimal nephrin promoter NPHS1 may comprise or consist of the nucleotide sequence shown as SEQ ID NO: 10, or a variant which is at least 70% identical to SEQ ID NO: 10.

Suitably, the viral vector is an adeno-associated virus (AAV). Suitably, the AAV vector is in the form of an AAV vector particle. In some embodimentsexamples, the AAV vector particle is a podocyte-specific AAV vector. In some embodimentsexamples, the AAV vector is AAV serotype 2/9, LK03 or 3B.

In some embodimentsexamples, the COL4A3, COL4A4 or COL4A5 transgene is a mini-gene.

In some embodimentsexamples, the viral vector additionally comprises a Woodchuck hepatitis post-transcriptional regulatory element (WPRE). In some embodimentsexamples, the viral vector does not comprise Woodchuck hepatitis post-transcriptional regulatory element (WPRE).

In some embodimentsexamples, the viral vector additionally comprises a Kozak sequence between the promoter and the COL4A3, COL4A4 or COL4A5 transgene.

Suitably, the viral vector additionally comprises a polyadenylation signal such as bovine growth hormone (bGH) polyadenylation signal or an early SV40 polyadenylation signal. In some embodimentsexamples, the polyadenylation signal is an early SV40 polyadenylation signal.

In one aspect, the present invention provides a viral vector gene therapy, wherein the viral vector comprises a COL4A3, COL4A4 or COL4A5 transgene.

In preferred ~~embodiments~~examples, the viral vector is a viral vector according to the present ~~invention~~disclosure.

In one aspect, the present ~~invention~~disclosure provides a viral vector gene therapy, wherein the gene therapy comprises:

a first viral vector comprising at least a portion of a COL4A3, COL4A4 or COL4A5 transgene; and

a second viral vector comprising at least a portion of a corresponding COL4A3, COL4A4 or COL4A5 transgene.

In preferred ~~embodiments~~examples, the first viral vector is a viral vector according to the present ~~invention~~disclosure, and/or the second viral vector is a viral vector according to the present ~~invention~~disclosure.

In one aspect, the present ~~invention~~disclosure provides a use of a viral vector or viral vector gene therapy as disclosed herein in the manufacture of a medicament for ~~according to the present invention, for use in treating or preventing Alport Syndrome.~~

Suitably, the viral vector or viral vector gene therapy is administered to a human patient. In some ~~embodiments~~examples, the viral vector or viral vector gene therapy is administered systemically. In some ~~embodiments~~examples, the viral vector or viral vector gene therapy is administered by intravenous injection. In some ~~embodiments~~examples, the viral vector or viral vector gene therapy is administered by injection into the renal artery.

Viral vectors

Adeno-associated viral (AAV) vectors

The viral vector may be an adeno-associated virus (AAV) and suitable AAV vector serotypes include 2/9, LK03 and 3B.

The viral vector may be in the form of an AAV vector particle.

CLAIMS

1. A viral vector, wherein the viral vector comprises a COL4A3, COL4A4 or COL4A5 transgene.
2. A viral vector according to claim 1, wherein:
 - (i) the COL4A3 transgene encodes a COL4A3 polypeptide which comprises or consists of the polypeptide sequence having at least 70% identity to SEQ ID NO: 1, or a fragment thereof;
 - (ii) the COL4A4 transgene encodes a COL4A4 polypeptide which comprises or consists of the polypeptide sequence having at least 70% identity to SEQ ID NO: 2, or a fragment thereof; and/or
 - (iii) the COL4A5 transgene encodes a COL4A5 polypeptide which comprises or consists of the polypeptide sequence having at least 70% identity to SEQ ID NO: 3, or a fragment thereof.
3. A viral vector according to claim 1 or 2, wherein: (i) the COL4A3 transgene encodes a full-length COL4A3 polypeptide, (ii) the COL4A4 transgene encodes a full-length COL4A4 polypeptide; and/or (iii) the COL4A5 transgene encodes a full-length COL4A5 polypeptide.
4. A viral vector according to any of the preceding claims, wherein the viral vector comprises a podocyte-specific promoter.
5. A viral vector according to any of the preceding claims, wherein the podocyte-specific promoter is minimal nephrin promoter NPHS1 or podocin promoter NPHS2, preferably wherein the podocyte-specific promoter is minimal nephrin promoter NPHS1.
6. A viral vector according to claim 5, wherein the minimal nephrin promoter NPHS1 comprises or consists of the nucleotide sequence shown as SEQ ID NO: 10, or a variant which is at least 70% identical to SEQ ID NO: 10.
7. A viral vector according to any of the preceding claims, wherein the viral vector is an adeno-associated virus (AAV).

8. A viral vector according to claim 7, wherein the AAV vector is in the form of an AAV vector particle.
9. A viral vector according to claim 7 or 8, wherein the AAV vector particle is a podocyte-specific AAV vector.
10. A viral vector according to any of claims 7 to 9, wherein the AAV vector is AAV serotype 2/9, LK03 or 3B.
11. A viral vector according to any of the preceding claims, wherein the COL4A3, COL4A4 or COL4A5 transgene is a mini-gene.
12. A viral vector according to any of the preceding claims, wherein the viral vector additionally comprises a Woodchuck hepatitis post-transcriptional regulatory element (WPRE).
13. A viral vector according to any of claims 1 to 11, wherein the viral vector does not comprise Woodchuck hepatitis post-transcriptional regulatory element (WPRE).
14. A viral vector according to any of the preceding claims, wherein the COL4A3, COL4A4 or COL4A5 transgene is human and/or comprises a hemagglutinin (HA) tag.
15. A viral vector according to any of the preceding claims, wherein the viral vector additionally comprises a Kozak sequence between the promoter and the COL4A3, COL4A4 or COL4A5 transgene.
16. A viral vector according to any of the preceding claims, wherein the viral vector additionally comprises a polyadenylation signal such as bovine growth hormone (bGH) polyadenylation signal or an early SV40 polyadenylation signal.
17. A viral vector according to claim 16, wherein the polyadenylation signal is an early SV40 polyadenylation signal.
18. A viral vector gene therapy, wherein the gene therapy comprises:

a first viral vector comprising at least a portion of a COL4A3, COL4A4 or COL4A5 transgene; and

a second viral vector comprising at least a portion of a corresponding COL4A3, COL4A4 or COL4A5 transgene.

19. A viral vector gene therapy according to claim 18, wherein the first viral vector is a viral vector as defined in any of claims 1 to 17 and/or the second viral vector is a viral vector as defined in any of claims 1 to 17.

20. Use of aA viral vector or viral vector gene therapy according to any of the preceding claims in the manufacture of a medicament for, ~~for use in~~ treating or preventing Alport Syndrome.

21. The use of aA viral vector or viral vector gene therapy ~~for use~~ according to claim 20, wherein the viral vector or viral vector gene therapy is to be administered to a human patient.

22. The use of aA viral vector or viral vector gene therapy ~~for use~~ according to claims 20 or 21, wherein the viral vector or viral vector gene therapy is to be administered systemically.

23. The use of aA viral vector or viral vector gene therapy ~~for use~~ according to any of claims 20 to 22, wherein the viral vector or viral vector gene therapy is to be administered by intravenous injection.

24. The use of aA viral vector or viral vector gene therapy ~~for use~~ according to any of claims 20 to 23, wherein the viral vector or viral vector gene therapy is to be administered by injection into the renal artery.