promoters this tropism defines the site of gene expression. Whereas in combination with cell-type specific promoters the degree of site-specificity (i.e. transgene expression only in rod or cone photoreceptors) is defined by the combination of both, the tropism of the AAV serotype and the specificity of the promoter (Schön et al. (2015) “Retinal gene delivery by adeno-associated virus (AAV) vectors: Strategies and applications” European journal of pharmaceutics and biopharmaceutics). Administration of AAVs into the eye's vitreous results in transduction of cells within the inner retina, mainly of ganglion cells and Müller glial cells (Trapani et al. (2014) “Vector platforms for gene therapy of inherited retinopathies” Prog Retin Eye Res).

[8] None of the naturally occurring AAV serotypes is capable of transducing photoreceptors when administered intravitreally because of the physical barrier at the inner limiting membrane (ILM) or the lack of appropriate receptors at the ILM.

[9] A prerequisite for successful gene therapy approaches is the efficient and long lasting transgene expression in the target cells with only minimal off-target expression and lowest possible adverse effects. Although, the available AAV vectors constitute valuable gene delivery tools there is still a strong demand for the development of improved AAVs.

[10] For targeting photoreceptors AAVs need to be administered into the subretinal space. This very invasive surgical procedure results in detachment of the retina from the RPE. Retinal detachment usually is temporal and the retina reattaches without any severe or long-term deleterious effects on retinal function and morphology. However, the risk for collateral damage due to the subretinal injection might be increased in the degenerated retina and/or if the detachment involves the foveo-macular region. Therefore, improved AAV vectors are needed, in particular regarding the bioavailability, route of delivery and the target cell specificity.

[11] Thus, there still exists a need for the development of further improved AAV vectors. Here, we describe novel AAV that evolved from an in vivo selection with improved characteristics for gene transfer into retinal cells and especially photoreceptor cells.

[12] The present invention complies with this need as described herein and as described in the Examples, Figures and claims.

SUMMARY

[13] In one aspect, the present disclosure refers to an adeno-associated virus (AAV), the AAV comprising an insertion of at least ~~6-8~~7 amino acids between the positions corresponding to position 587 and 588 of SEQ ID NO: 1; wherein the insertion sequence

has from the N-terminus to the C-terminus the formula I: X_{1A}-X_{1B}-X_2-X_3-X_4-X_5-X_6-X_7 (formula I) wherein X_5 is selected from P (Pro), L (Leu) and V (Val); wherein X_7 is R (Arg); wherein X_{1A} and/or X_{1B} is/are selected from G, S, N, V and P; wherein X_2 is selected from L, S and N; wherein X_3 is selected from S, P, T, Q; wherein X_4 is selected from P, G, S, A and T; wherein X_6 is selected from T, P, N, Q and S; and wherein X_{1A} and~~/or~~ X_{1B} are ~~independently optionally absent or~~ present or wherein X_{1A} is absent and X_{1B} is present or wherein X_{1B} is absent and X_{1A} is present, or wherein X_5 is selected from S (Ser) and T (Thr); wherein X_7 is S (Ser); wherein X_{1A} and/or X_{1B} is/are selected from G, R, H and S; wherein X_2 is selected from A, S and Q; wherein X_3 is selected from H, N and A; wherein X_4 is selected from R, Q and D; wherein X_6 is selected from R, S and D; and wherein X_{1A} and~~/or~~ X_{1B} are ~~independently optionally absent or~~ present or wherein X_{1A} is absent and X_{1B} is present or wherein X_{1B} is absent and X_{1A} is present, or wherein X_5 is selected from A (Ala) and Q (Gln); wherein X_7 is A (Ala); wherein X_{1A} and/or X_{1B} is/are selected from N and R; wherein X_2 is selected from S and G; wherein X_3 is selected from R and S; wherein X_4 is selected from P and L; wherein X_6 is selected from A and N; and wherein X_{1A} and~~/or~~ X_{1B} are ~~independently optionally absent or~~ present or wherein X_{1A} is absent and X_{1B} is present or wherein X_{1B} is absent and X_{1A} is present, wherein viral AAV DNA is present in mouse retinal nuclear extracts after 24 hours, when the AAV is to be administered intravenously into the tail vein of a C57-Bl6J mouse, and wherein viral AAV DNA is present in MAC-sorted rods using anti-CD73-coated magnetic beads and/or FACS-sorted cones, wherein the cones express eGFP and are FACS sorted based on their eGFP expression, 24 hours after administration, when the AAV is to be administered intravenously into the tail vein of a C57-Bl6J mouse for rod sorting and into the tail vein of RG-eGFP mice line R685933 for cone sorting.

[14] In another aspect, the present disclosure relates to an AAV of the present disclosure for use as a medicament, wherein the AAV comprises a transgene, a molecule or a polypeptide.

[15] In addition, the present disclosure relates to an AAV of the present disclosure for use in treating a photoreceptor cell disease, wherein the AAV comprises a transgene, a molecule or a polypeptide. In yet another aspect, the present disclosure relates to use of the AAV of the present disclosure in the manufacture of a medicament for treating a photoreceptor cell disease

[16] In yet another aspect, the present disclosure further concerns pharmaceutical composition comprising an AAV of the present disclosure.

[17] In yet another aspect, the present disclosure relates to an in vitro use of an AAV of the present

disclosure for transduction of the nucleus of retinal cells.

[18] Furthermore, the present disclosure relates to a method for screening an insertion sequence, the method comprising i) intravenously administering an AAV library, wherein each AAV comprises an insertion sequence, into a subject; ii) isolating viral AAV DNA from retinal nuclear extracts; iii) subcloning the isolated viral AAV DNA into a second AAV library; iv) intravenously administering the second AAV library as obtained in step iii) into a subject; v) isolating viral AAV DNA from rod or cone photoreceptors; vi) determining the sequence of the insertion sequence, thereby obtaining the insertion sequence.

[18a] In yet another aspect, the present disclosure relates to a method for screening an insertion sequence, the method comprising

i) an AAV library to be intravenously administered into a subject, wherein each AAV comprises an insertion sequence of at least 7 amino acids between the positions corresponding to position 578 and 588 of SEQ ID NO: 1;

ii) isolating viral AAV DNA from retinal nuclear extracts;

iii) subcloning the isolated viral AAV DNA into a second AAV library;

iv) the second AAV library as obtained in step iii) to be intravenously administered into a subject;

v) isolating viral DNA from rod or cone photoreceptors;

vi) determining the sequence of the insertion sequence, thereby obtaining the insertion sequence.

[19] In yet another aspect, the present disclosure additionally relates to a peptide (insertion sequence) obtainable by the method of the present disclosure, wherein the peptide has from the N-terminus to the C-terminus the formula I: X_{1A}-X_{1B}-X_2-X_3-X_4-X_5-X_6-X_7 (formula I), wherein X_5 is selected from P (Pro), L (Leu) and V (Val); wherein X_7 is R (Arg); wherein X_{1A}, X_{1B}, X_2, X_3, X_4, X_6 are independently any amino acid; wherein X_{1A} and/or X_{1B} are independently optionally absent or present, or wherein X_5 is selected from S (Ser) and T (Thr); wherein X_7 is S (Ser); wherein X_{1A}, X_{1B}, X_2, X_3, X_4, X_6 are independently any amino acid; wherein X_{1A} and/or X_{1B} are independently optionally absent or present, or wherein X_5 is selected from A (Ala) and Q (Gln); wherein X_7 is A (Ala); wherein X_{1A}, X_{1B}, X_2, X_3, X_4, X_6 are independently any amino acid; wherein X_{1A} and/or X_{1B} are independently optionally absent or present.

[20] In yet another aspect, the present disclosure also relates to a kit comprising an AAV of the present disclosure.

BRIEF DESCRIPTION OF FIGURES

[21] The figures show:

[22] **Figure 1: Peptide insertions from whole retina**

Claims

1. An adeno-associated virus (AAV), the AAV comprising

an insertion of at least ~~6~~ 7 amino acids between the positions corresponding to position 587 and 588 of SEQ ID NO: 1, wherein the insertion sequence has from the N-terminus to the C-terminus the formula I:

X_{1A}-X_{1B}-X_2-X_3-X_4-X_5-X_6-X_7 (formula I)

wherein X_5 is selected from P (Pro), L (Leu) and V (Val);

wherein X_7 is R (Arg);

wherein X_{1A} and/or X_{1B} is/are selected from G, S, N, V and P;
wherein X_2 is selected from L, S and N;
wherein X_3 is selected from S, P, T, Q;
wherein X_4 is selected from P, G, S, A and T;
wherein X_6 is selected from T, P, N, Q and S; and
wherein X_{1A} and~~/or~~ X_{1B} are ~~independently optionally absent or~~ present or wherein X_{1A} is absent and X_{1B} is present or wherein X_{1B} is absent and X_{1A} is present,

or

wherein X_5 is selected from S (Ser) and T (Thr);

wherein X_7 is S (Ser);

wherein X_{1A} and/or X_{1B} is/are selected from G, R, H and S;
wherein X_2 is selected from A, S and Q;
wherein X_3 is selected from H, N and A;
wherein X_4 is selected from R, Q and D;
wherein X_6 is selected from R, S and D; and
wherein X_{1A} and~~/or~~ X_{1B} are ~~independently optionally absent or~~ present or wherein X_{1A} is absent and X_{1B} is present or wherein X_{1B} is absent and X_{1A} is present,

or

wherein X_5 is selected from A (Ala) and Q (Gln);

wherein X_7 is A (Ala);

wherein X_{1A} and/or X_{1B} is/are selected from N and R;

wherein X_2 is selected from S and G;

wherein X_3 is selected from R and S;

wherein X_4 is selected from P and L;

wherein X_6 is selected from A and N; and

wherein X_{1A} and~~/or~~ X_{1B} are ~~independently optionally absent or~~ present or wherein X_{1A} is absent and X_{1B} is present or wherein X_{1B} is absent and X_{1A} is present,

wherein viral AAV DNA is present in mouse retinal nuclear extracts after 24 hours, when the AAV is to be administered intravenously into the tail vein of a C57-Bl6J mouse, and

wherein viral AAV DNA is present in MAC-sorted rods using anti-CD73-coated magnetic beads and/or FACS-sorted cones, wherein the cones express eGFP and are FACS sorted based on their eGFP expression, 24 hours after administration, when the AAV is to be administered intravenously into the tail vein of a C57-Bl6J mouse for rod sorting and into the tail vein of RG-eGFP mice line R685933 for cone sorting.

~~2. The AAV of claim 1, wherein X_{1A} is absent and X_{1B} is present or wherein X_{1B} is absent and X_{1A} is present.~~

~~3~~2. The AAV of claim 1~~ or 2~~, wherein the insertion sequence has from the N-terminus to the C-terminus the formula II:

$$L_3\text{-}L_2\text{-}L_1\text{-}X_{1A}\text{-}X_{1B}\text{-}X_2\text{-}X_3\text{-}X_4\text{-}X_5\text{-}X_6\text{-}X_7\text{-}L_4\text{-}L_5\text{-}L_6 \text{ (formula II)}$$

wherein L is a flexible amino acid; and

wherein L_1, L_2, L_3, L_4, L_5 and L_6 are independently selected from Ala, Leu, Gly, Ser and Thr; and

wherein L_1, L_2, L_3, L_4, L_5 and L_6 are independently optionally absent or present.

~~43~~<u>3</u>. The AAV of claim ~~32~~<u>2</u>, wherein X_5 is selected from P (Pro), L (Leu) and V (Val);
wherein X_7 is R (Arg);
wherein X_{1A} and/or X_{1B} is/are selected from G, S, N, V and P;
wherein X_2 is selected from L, S and N;
wherein X_3 is selected from S, P, T, Q;
wherein X_4 is selected from P, G, S, A and T;
wherein X_6 is selected from T, P, N, Q and S and wherein L_1, L_2, L_3, L_4, L_5 and L_6 are present; and
~~wherein X_{1A} and/or X_{1B} are independently optionally absent or present; and~~
wherein the AAV is preferably AAV2.

~~54~~<u>4</u>. The AAV of claim ~~32~~<u>2</u>, wherein one of X_{1A} or X_{1B} is present and the other one of X_{1A} or X_{1B} is absent, and

wherein all of L_1 , L_2, L_3, L_4 and L_5 are present, and wherein L_6 is absent.

~~6. The AAV of any one of the preceding claims, wherein X_{1A} and/or X_{1B} and/or X_2 and/or X_3 is not an acidic amino acid.~~

~~7. The AAV of any one of the preceding claims, wherein one of X_{1A} or X_{1B} is present and the other one of X_{1A} or X_{1B} is absent and wherein X_2 is not a basic or acidic amino acid.~~

~~8. The AAV of any one of the preceding claims, wherein X_{1A} and/or X_{1B} and/or X_2 and/or X_3 and/or X_4 and/or X_6 is not a hydrophobic-aromatic amino acid.~~

<u>5. The AAV of any one of claims 1 to 4, wherein the AAV comprises the insertion sequence GLSPPTR (SEQ ID NO: 10), SSPGLPR (SEQ ID NO: 11), NSTSVNR</u>

(SEQ ID NO: 12), VSSSLQR (SEQ ID NO: 13), PNQAPPR (SEQ ID NO: 14) or NNPTPSR (SEQ ID NO: 15).

6. The AAV of claim 5, wherein the AAV comprises the insertion sequence GLSPPTR (SEQ ID NO: 10) or NNPTPSR (SEQ ID NO: 15).

7. The AAV of claim 1 or 2, wherein the AAV comprises the insertion sequence GAHRSDS (SEQ ID NO: 16), RANQTSS (SEQ ID NO: 17), HSARTDS (SEQ ID NO: 18) or SQNDSRS (SEQ ID NO: 19).

8. The AAV of claim 1 or 2, wherein the AAV comprises the insertion sequence NSRPAAA (SEQ ID NO: 20) or RGSLQNA (SEQ ID NO: 21).

9. The AAV of any one of the preceding claims for use as a medicament, wherein the AAV comprises a transgene, a molecule or a polypeptide.

10. Use of the AAV of any one of the preceding claims in the manufacture of a medicament for treating a photoreceptor cell disease, wherein the AAV comprises a transgene, a molecule or a polypeptide.

11. Pharmaceutical composition comprising an AAV of any one of claims 1-8.

12. In vitro use of an AAV of any one of claims 1-8 for transduction of the nucleus of retinal cells.

13. A method for screening an insertion sequence, the method comprising

i) an AAV library to be intravenously administered into a subject, wherein each AAV comprises an insertion sequence of at least 7 amino acids between the positions corresponding to position 578 and 588 of SEQ ID NO: 1;

ii) isolating viral AAV DNA from retinal nuclear extracts ;

iii) subcloning the isolated viral AAV DNA into a second AAV library;

iv) the second AAV library as obtained in step iii) to be intravenously administered into a subject;

v) isolating viral DNA from rod or cone photoreceptors;

vi) determining the sequence of the insertion sequence,

thereby obtaining the insertion sequence.

~~14. A peptide (insertion sequence) obtainable by the method of claim 13, wherein the peptide has from the N-terminus to the C-terminus the formula I:~~

~~X_{1A}-X_{1B}-X_2-X_3-X_4-X_5-X_6-X_7 (formula I)~~

~~wherein X_5 is selected from P (Pro), L (Leu) and V (Val);~~
~~wherein X_7 is R (Arg);~~
~~wherein X_{1A}, X_{1B}, X_2, X_3, X_4, X_6 are independently any amino acid;~~
~~wherein X_{1A} and/or X_{1B} are independently optionally absent or present,~~
~~or~~
~~wherein X_5 is selected from S (Ser) and T (Thr);~~
~~wherein X_7 is S (Ser);~~
~~wherein X_{1A}, X_{1B}, X_2, X_3, X_4, X_6 are independently any amino acid;~~
~~wherein X_{1A} and/or X_{1B} are independently optionally absent or present,~~
~~or~~
~~wherein X_5 is selected from A (Ala) and Q (Gln);~~
~~wherein X_7 is A (Ala);~~
~~wherein X_{1A}, X_{1B}, X_2, X_3, X_4, X_6 are independently any amino acid;~~
~~wherein X_{1A} and/or X_{1B} are independently optionally absent or present.~~

~~15~~14. Kit comprising an AAV of any one of claims 1-8.