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ADENO-ASSOCIATED VIRUS COMPOSITIONS HAVING INCREASED BRAIN ENRICHMENT AND/OR HEART ENRICHMENT

Abstract

Described herein are compositions and kits comprising recombinant adeno-associated viruses (rAAVs) with increased transduction enrichment in the brain, heart, or brain and heart. The rAAV compositions described herein encapsidate a transgene, such as a therapeutic nucleic acid. Gene therapy using the rAAVs is described. Also described are methods of treating brain-related and heart-related diseases and conditions.

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Background/Summary

BACKGROUND

[0001] Recombinant adeno-associated viruses (rAAVs) are widely used as vectors for gene delivery in therapeutic applications because of their ability to transduce both dividing and non-dividing cells, their long-term persistence as episomal DNA in infected cells, and their low immunogenicity. These characteristics make them appealing for applications in therapeutic applications, such as gene therapy. However, there is a need to significantly improve the performance of existing AAV serotypes to selectively and efficiently express in distinct cell-types, upon systemic delivery to a subject. This need is especially acute when the AAV must be expressed in the brain or heart.

SUMMARY OF THE INVENTION

[0002] Disclosed herein are rAAVs with peptide insertions and substitutions engineered into the capsid structure through iterative rounds of selection in non-human primates (NHPs), yielding variants having increased transduction when measured in the brain, heart, or brain and heart relative to the wild type rAAV on which the variant is based.

[0003] The present invention provides rAAVs with widespread transduction to the brain, heart, or brain and heart. Following IV injection, unmodified rAAVs such as those derived from AAV9 (SEQ ID NO: 1) may not have sufficient tissue enrichment to treat many human diseases by delivery of an AAV cargo Directed evolution of AAV9 as described herein has provided modified rAAVs that exhibit increased viral tissue enrichment in the brain. Accordingly, engineered rAAVs described herein are particularly useful in delivering DNA cargo to brain tissue and/or heart tissue.

[0004] The present invention provides a mutant Adeno-Associated Virus (AAV) capsid protein comprising at least 95% sequence identity to the wild-type AAV capsid protein, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-1496.

[0005] The mutant AAV capsid protein may comprise a 7-mer amino acid insertion sequence located between residues 588 and 589 in relation to the wild-type AAV capsid protein. The mutant AAV capsid protein may comprise at least a 95% sequence identity to the wild-type Adeno-Associated Virus serotype 9 (AAV9) capsid of SEQ ID NO: 1. In the AAV9 capsid, the amino acids immediately preceding the 7-mer amino acid insertion sequence are -AQ-. The amino acids immediately following the 7-mer amino acid insertion sequence are also typically -AQ-.

[0006] In aspects of the invention, 60 copies of the AAV capsid protein may be assembled into the AAV capsid. The AAV capsid protein may present in VP1, VP2, and VP3 of the mutant AAV capsid.

[0007] The mutant AAV capsid protein may comprise a sequence selected from the group consisting of SEQ ID NO: 2-898. Advantageously, the AAV capsid protein may be characterized with increased transduction enrichment relative to the wild-type AAV9 when measured in brain tissue in a subject when delivered to the subject systemically. In aspects of the invention, the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-594.

[0008] The mutant AAV capsid protein may comprise a sequence selected from the group consisting of SEQ ID NO: 595-1496. Advantageously, the mutant AAV capsid protein may be

characterized by an increased transduction enrichment relative to the wild-type AAV9 when measured in heart tissue in a subject when delivered to the subject systemically. In aspects of the invention, the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 899-1496.

[0009] The mutant AAV capsid protein may comprise a sequence selected from the group consisting of SEQ ID NO: 595-898. The mutant AAV capsid protein may be characterized by an increased transduction enrichment relative to the wild-type AAV9 when measured in brain tissue and heart tissue in a subject when delivered to the subject systemically.

[0010] In aspects of the invention the AAV capsid protein is integrated into an AAV capsid. The AAV capsid is chimeric. The AAV capsid may be isolated and purified.

[0011] Aspects of the invention further provide a composition comprising the AAV capsid of the invention, wherein the AAV capsid comprises an AAV capsid protein comprising at least 95% sequence identity to the wild-type AAV capsid protein, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-1496. The composition may be formulated for systematic administration to a subject. Systematic administration of the composition may result in expression of a gene product delivered by the AAV capsid. Administration of the composition results in increased transduction enrichment relative to the wild-type AAV9 when measured in heart and/or brain.

[0012] Aspects of the invention further provide a method of treating a disease or condition in a subject comprising administering a therapeutically effective amount of the composition of the invention. For example, provided is a composition for use in treating a disease or condition in a subject, the use comprising administering a therapeutically effective amount of a composition of the invention. Advantageously, because the compositions of the invention comprise AAV capsid proteins that are direct engineered AAVs to brain tissue, heart tissue, or brain and heart tissues 10 specifically, the compositions provide effective methods and uses for treating diseases associated with the brain, heart, or brain and heart.

TABLE-US-00001 TABLE 1 Brain Targeting sequences SEQ ID AA sequence NO: (#587-597) 2 AQAAATAAQAQ 3 AQAALPPRSAQ 4 AQAASSGHSAQ 5 AQAESRTSAAQ 6 AQAFSVSPDAQ 7 AQAGGHSNNAQ 8 AQAGPSNAMAQ 9 AQAGSIRDDAQ 10 AQAHGDGSLAQ 11 AQAHSSGVGAQ 12 AQAHTHTLSAQ 13 AQAIAGYASAQ 14 AQAISNTTVAQ 15 AQALARDHSAQ 16 AQALRGSLCAQ 17 AQAMDRTNMAQ 18 AQAMQTRNPAQ 19 AQAMSSLTAAQ 20 AQAPGALMTAQ 21 AQAPREIAIAQ 22 AQAQADRPDAQ 23 AQAQGNLAFAQ 24 AQARSNEMTAQ 25 AQASGHAPTAQ 26 AQASGSNAHAQ 27 AQASLAVTSAQ 28 AQASRDSGSAQ 29 AQASRSESYAQ 30 AQASTRVVNAQ 31 AQATSDRPTAQ 32 AQATVTHKDAQ 33 AQAVGTMAPAQ 34 AQDASRVLAAQ 35 AQDMTGLVIAQ 36 AQDNRTRTNAQ 37 AQDPSRSASAQ 38 AQDPTTRNEAQ 39 AQDRSNITLAQ 40 AQDSAQNRGAQ 41 AQDSGPSKAAQ 42 AQDSIQYSFAQ 43 AQDSREQGRAQ 44 AQDSRLGVTAQ 45 AQDSRQPPNAQ 46 AQDSRVPSQAQ 47 AQDSVNLSSAQ 48 AQDVRHGGTAQ 49 AQDVRSNTSAQ 50 AQDVSVSAFAQ 51 AQDYAYRTGAQ 52 AQEHTRGETAQ 53 AQEITHSHSAQ 54 AQENHTENKAQ 55 AQENRVANKAQ 56 AQEPERFMFAQ 57 AQEPRLATDAQ 58 AQERTASLHAQ 59 AQESNRHPYAQ 60 AQESSHTGGAQ 61 AQESVGRGEAQ 62 AQEVGGGSKAQ 63 AQFALPVPSAQ 64 AQFGSVQDRAQ 65 AQFGTGGVPAQ 66 AQFGTMGDKAQ 67 AQFGVTAGSAQ 68 AQFINTVPVAQ 69 AQFLVGGTVAQ 70 AQFSAFLPAAQ 71 AQFSGMSNSAQ 72 AQFVKIAKSAQ 73 AQFVTLVTSAQ 74 AQGADARLHAQ 75 AQGDAARSGAQ 76 AQGGGSHSSAQ 77 AQGHQHVTEAQ 78 AQGHSETRLAQ 79 AQGIGHMNDAQ 80 AQGLALTLNAQ 81 AQGLAVASPAQ 82 AQGLSSPLAAQ 83 AQGLVLSSTAQ 84 AQGLVSSSVAQ 85 AQGLYNIRSAQ 86 AQGNAVSVAAQ 87 AQGNHTRHAAQ 88 AQGNLTRLTAQ 89 AQGNNTRAHAQ 90 AQGNNTTSRAQ 91 AQGNQSNAAAQ 92 AQGNRTRAPAQ 93 AQGNRTTNDAQ 94

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TABLE-US-00002 TABLE 2 Brain and Heart Sequences SEQ ID AA sequence NO: (#587-597) 595 AQAAGVSAGAQ 596 AQAGHNMGGAQ 597 AQANKVKDLAQ 598 AQANLTRVTAQ 599 AQANNTMKQAQ 600 AQANQTRPFAQ 601 AQANSVKNIAQ 602 AQANTTKSSAQ 603 AQANTTRQTAQ 604 AQANTVRNTAQ 605 AQARAAPQTAQ 606 AQASAGSSGAQ 607 AQASNHTSGAQ 608 AQASRSEIGAQ 609 AQASSSAREAQ 610 AQATGYSGAAQ 611 AQATHSREQAQ 612 AQATNSAVGAQ 613 AQDARPSPNAQ 614 AQDNHVRTVAQ 615 AQDNKVKTFAQ 616 AQDNMIRSIAQ 617 AQDNSTRISAQ 618 AQDNSVKHSAQ 619 AQDNSVRLTAQ 620 AQDNSVRPSAQ 621 AQDNSVRSPAQ 622 AQDNVIRSIAQ 623 AQDRAQSHSAQ 624 AQDRSQSHSAQ 625 AQDTSHRASAQ 626 AQEGGTGKVAQ 627 AQENHVRSSAQ 628 AQENKIKTNAQ 629 AQENNTRMVAQ 630 AQENQVRNTAQ 631 AQENRIINHAQ 632 AQENSTKSMAQ 633 AQENSTRVQAQ 634 AQENTTKHLAQ 635 AQENTTKSLAQ 636 AQENTVRNMAQ 637 AQETAVRSSAQ 638 AQETHNRAGAQ 639 AQEVRGGTMAQ 640 AQFNSTKVVAQ 641 AQFNSTRSVAQ 642 AQFNSVRPVAQ 643 AQGGAPPTNAQ 644 AQGNAIRNGAQ 645 AQGNAVKPFAQ 646 AQGNGTRGFAQ 647 AQGNGTRPTAQ 648 AQGNITKVFAQ 649 AQGNNSKPAAQ 650 AQGNNTKQMAQ 651 AQGNNTRLTAQ 652 AQGNQLKNTAQ 653 AQGNRIAQVAQ 654 AQGNRKPNEAQ 655 AQGNRTQPSAQ 656 AQGNRTSGGAQ 657 AQGNRVQNVAQ 658 AQGNSIINHAQ 659 AQGNSSKNLAQ 660 AQGNSVKDSAQ 661 AQGNSVKGTAQ 662 AQGNSVRISAQ 663 AQGNSVRTVAQ 664 AQGNTIKDYAQ 665 AQGNTTRHPAQ 666 AQGNTTRVPAQ 667 AQGNTVKSEAQ 668 AQGRNTSMEAQ 669 AQGRSSQGEAQ 670 AQGSRGSGAAQ 671 AQGTRSTDNAQ 672 AQGTSSNTGAQ 673 AQGVASSHQAQ 674 AQHGSSTLGAQ 675 AQHGSTFTGAQ 676 AQHIGSIPAAQ 677 AQHNNVRPVAQ 678 AQHNSTRNAAQ 679 AQHNTTKPIAQ 680 AQHSQSSKDAQ 681 AQHTSSISSAQ 682 AQIAHASSMAQ 683 AQIAHSSSGAQ 684 AQIISSNVPAQ 685 AQINATRNQAQ 686

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AQNSHNVPSAQ 755 AQNSNNVAGAQ 756 AQNSRSVSDAQ 757 AQNSSTRTGAQ 758
AQNSTKPITAQ 759 AQNSTRPLTAQ 760 AQNTRGLVEAQ 761 AQNTTKGMAAQ 762
AQNTTRMTTAQ 763 AQNTTRSESAQ 764 AQNVRNVLDAQ 765 AQPAGRNLDAQ 766
AQPGYGNQGAQ 767 AQPMSRDTSAQ 768 AQPNAVKPMAQ 769 AQPNITRNNAQ 770
AQPNIVRSTAQ 771 AQPNQTKTTAQ 772 AQPNTTKLSAQ 773 AQPNTTKPIAQ 774
AQPNTTRSTAQ 775 AQPNVTRGFAQ 776 AQPNVTRTVAQ 777 AQQATVRTEAQ 778
AQQDRSSAVAQ 779 AQQHGVSAPAQ 780 AQQIQRSIDAQ 781 AQQNAIRQSAQ 782
AQQNATKGIAQ 783 AQQNLTRMTAQ 784 AQQNNVRPSAQ 785 AQQNSIRSVAQ 786
AQQNSTRSLAQ 787 AQQNTTRTSAQ 788 AQQNYTKVAAQ 789 AQQRIPSNTAQ 790
AQQRTSSQEAQ 791 AQQSSSRLGAQ 792 AQQVRSDMGAQ 793 AQRDGLILIAQ 794
AQRDNIILIAQ 795 AQRDSTMHAAQ 796 AQRDTHVFHAQ 797 AQRGDLHFQAQ 798
AQRLSQSSDAQ 799 AQRSGLILFAQ 800 AQRSGSHFVAQ 801 AQRVGQSVSAQ 802
AQRVVGPLFAQ 803 AQSDRGNHSAQ 804 AQSDSRNASAQ 805 AQSESARGNAQ 806
AQSGHNASGAQ 807 AQSGSHTNSAQ 808 AQSGSSRGEAQ 809 AQSLSQPERAQ 810
AQSMGHSHAAQ 811 AQSMSYGSSAQ 812 AQSNAAKPVAQ 813 AQSNATTRAAQ 814
AQSNDIRNNAQ 815 AQSNDTKNTAQ 816 AQSNGIRNTAQ 817 AQSNHTTRSAQ 818
AQSNLVRQTAQ 819 AQSNNAKPSAQ 820 AQSNNSSRPAQ 821 AQSNQTRGNAQ 822
AQSNSTKMIAQ 823 AQSNSTKNAAQ 824 AQSNSTKNVAQ 825 AQSNSTKSLAQ 826
AQSNSTRSSAQ 827 AQSNSVKAPAQ 828 AQSNSVKERAQ 829 AQSNSVKITAQ 830
AQSNSVKSFAQ 831 AQSNSVKTVAQ 832 AQSNSVRLVAQ 833 AQSNTTKPIAQ 834
AQSNTTRPMAQ 835 AQSNTTRQTAQ 836 AQSNTTTRAAQ 837 AQSNTVKAMAQ 838
AQSNTVKNAAQ 839 AQSNTVRNSAQ 840 AQSNTVRSSAQ 841 AQSNVIRNSAQ 842
AQSNYTRSPAQ 843 AQSPSRHTEAQ 844 AQSQHASSAAQ 845 AQSQSSSGSAQ 846
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AQSTAIRQDAQ 851 AQSTQTRAAAQ 852 AQSTQVRSEAQ 853 AQSTRGNDNAQ 854
AQSTSTHGAAQ 855 AQTGVRSGDAQ 856 AQTMRQTNDAQ 857 AQTNHTREVAQ 858
AQTNHVKTMAQ 859 AQTNHVRSAAQ 860 AQTNLVKPTAQ 861 AQTNMTRTTAQ 862
AQTNNATRPAQ 363 AQTNNAVRAAQ 864 AQTNNTKSTAQ 865 AQTNNTLRSAQ 866
AQTNNTSPGAQ 867 AQTNNVRAPAQ 868 AQTNSIKSTAQ 869 AQTNSIRPIAQ 870
AQTNSVKLIAQ 871 AQTNTTKSMAQ 872 AQTNTVRASAQ 873 AQTNTVRQTAQ 874
AQTRGASNEAQ 875 AQTSGRSDAAQ 876 AQTVSDRSGAQ 877 AQVGHSAVSAQ 878
AQVHNRADVAQ 879 AQVHSGPVSAQ 880 AQVNATRNVAQ 881 AQVNHTKGLAQ 882
AQVNSLGNNAQ 883 AQVNSTKSMAQ 884 AQVNSTKSTAQ 885 AQVNSTRGNAQ 886

AQVNTTKPIAQ 887 AQVNTTQSGAQ 888 AQVSDTRAGAQ 889 AQVSHSLASAQ 890 AQVSSSGMTAQ 891 AQVSVTRSDAQ 892 AQWAANTSGAQ 893 AQYAGVSSPAQ 894 AQYGSHGVGAQ 895 AQYNSTKNGAQ 896 AQYNSTKNMAQ 897 AQYNSTKSHAQ 898 AQYNTTKPIAQ

TABLE-US-00003 TABLE 3 Heart Sequences SEQ ID AA sequence NO: (#587-597) 899 AQAAHNKDSAQ 900 AQADARNTSAQ 901 AQAESRGSAAQ 902 AQAESRIGSAQ 903 AQAESRTMPAQ 904 AQAHAATSAAQ 905 AQAISLRGDAQ 906 AQAKGSTLLAQ 907 AQALARSVSAQ 908 AQALGQSRVAQ 909 AQALSHSSGAQ 910 AQANHTRVAAQ 911 AQAQGENLLAQ 912 AQARTGPMLAQ 913 AQARTHLQDAQ 914 AQARVGATAAQ 915 AQARVGTNVAQ 916 AQASHSRDAAQ 917 AQASSNKTAAQ 918 AQASSSTAGAQ 919 AQATARNAVAQ 920 AQATGRLPEAQ 921 AQATRNTDMAQ 922 AQATSIRGPAQ 923 AQATVSSRIAQ 924 AQAVNKPLSAQ 925 AQAVPQSAGAQ 926 AQAVPSHHGAQ 927 AQAVRGAMNAQ 928 AQAVSGIRIAQ 929 AQAVSRSVDAQ 930 AQAVWPTRNAQ 931 AQAYARGESAQ 932 AQAYGETFWAQ 933 AQCLGPCLQAQ 934 AQDGIRLTPAQ 935 AQDGRTVSMAQ 936 AQDGSTRMGAQ 937 AQDIRSTASAQ 938 AQDLSTARSAQ 939 AQDLSTRVAAQ 940 AQDMSRSTGAQ 941 AQDNVHSRGAQ 942 AQDQHNKSQAQ 943 AQDQKNATGAQ 944 AQDRAQSNMAQ 945 AQDRGTSQTAQ 946 AQDRRSSDLAQ 947 AQDRSHGHNAQ 948 AQDRSNTSNAQ 949 AQDRSTTHAAQ 950 AQDSLPEPYAQ 951 AQDSNRAVAAQ 952 AQDSNRPSNAQ 953 AQDSRQSKEAQ 954 AQDSSNRTGAQ 955 AQDSYTRSAAQ 956 AQDTHARGLAQ 957 AQDTRQSFSAQ 958 AQEASIRSGAQ 959 AQEGNISRPAQ 960 AQELTMPPYAQ 961 AQEMSKANMAQ 962 AQENQRSGAAQ 963 AQERGKNLEAQ 964 AQERGTVSTAQ 965 AQERNALREAQ 966 AQESGQRNQAQ 967 AQESNSYRPAQ 968 AQESRATTPAQ 969 AQESTKYHAAQ 970 AQESTRISGAQ 971 AQETGRYNPAQ 972 AQETTSWVFAQ 973 AQEVNRSSYAQ 974 AQEVSRGKDAQ 975 AQEYGKATAAQ 976 AQFAGMRTAAQ 977 AQFDSVRNSAQ 978 AQFGSRDGSAQ 979 AQFGSRQPEAQ 980 AQFHNTRSEAQ 981 AQFNSDRPGAQ 982 AQFNSTRIVAQ 983 AQFNVSSTGAQ 984 AQFQVATRDAQ 985 AQFSNRTASAQ 986 AQFSRGVNEAQ 987 AQGAGALRGAQ 988 AQGAGRTLEAQ 989 AQGDRLTHGAQ 990 AQGFGHSVSAQ 991 AQGGTAARGAQ 992 AQGGVRNAEAQ 993 AQGHHRTVEAQ 994 AQGHSANLGAQ 995 AQGIRNQAEAQ 996 AQGLGTSSNAQ 997 AQGLNRLTPAQ 998 AQGLRSSDSAQ 999 AQGMRTSNDAQ 1000 AQGNGSRMAAQ 1001 AQGNRIAREAQ 1002 AQGRSLTTDAQ 1003 AQGSKPSQMAQ 1004 AQGSYANSQAQ 1005 AQGVAKGNEAQ 1006 AQGVMGMRNAQ 1007 AQGVRSGLSAQ 1008 AQGVRSHDAAQ 1009 AQGVSNRANAQ 1010 AQGVSRATPAQ 1011 AQGVTLGSRAQ 1012 AQGYNSTPGAQ 1013 AQGYSGATGAQ 1014 AQGYSHSSGAQ 1015 AQHFVDSGLAQ 1016 AQHGTSRPDAQ 1017 AQHIVTHDLAQ 1018 AQHLVDRSLAQ 1019 AQHMSDSPHAQ 1020 AQHMVPSLLAQ 1021 AQHNLPVLHAQ 1022 AQHNPTRADAQ 1023 AQHPVQSSLAQ 1024 AQHQAKLQNAQ 1025 AQHQIAYDLAQ 1026 AQHQVPSLLAQ 1027 AQHRIATVEAQ 1028 AQHRVSEHFAQ 1029 AQHSSIGSSAQ 1030 AQHSVSHHFAQ 1031 AQHYLSTDFAQ 1032 AQIASRGLAAQ 1033 AQIDNRHNGAQ 1034 AQIGASSGGAQ 1035 AQIGGAVTTAQ 1036 AQIGGHERPAQ 1037 AQIGHVSANAQ 1038 AQIGRVTNEAQ 1039 AQIGSRASDAQ 1040 AQIGSSRLGAQ 1041 AQIHGKEREAQ 1042 AQIHLSSATAQ 1043 AQIHSRGDTAQ 1044 AQIHSTNPGAQ 1045 AQIISNRAVAQ 1046 AQILHLPMNAQ 1047 AQILTSTVGAQ 1048 AQINSTATGAQ 1049 AQIPNFPTLAQ 1050 AQIPSGNVSAQ 1051 AQIPSKSPAAQ 1052 AQIPSTHTGAQ 1053 AQIPSTRAGAQ 1054 AQIRHGATDAQ 1055 AQIRPRPEDAQ 1056 AQIRSDHSSAQ 1057 AQIRSDSAMAQ 1058 AQIRSSTGDAQ 1059 AQIRSSTIFAQ 1060 AQIRTMSSEAQ 1061 AQISNRTSSAQ 1062 AQISRQEVLAQ 1063 AQISSHSPGAQ 1064 AQISSSRLSAQ 1065 AQISVSLRSAQ 1066 AQITGVPMNAQ 1067 AQITRNADNAQ 1068 AQITSSTQFAQ 1069 AQITTRIEQAQ 1070 AQIVKPNITAQ 1071 AQIVSRTENAQ 1072 AQIVVRGDSAQ 1073 AQIYSASTPAQ 1074 AQKGQPPFLAQ 1075 AQKHHVPFDAQ 1076 AQKLDLPMTAQ 1077 AQKLLGGLSAQ 1078 AQKLSLPTTAQ 1079

AQKNGQPVQAQ 1080 AQKPYVPHDAQ 1081 AQKQRSEDPAQ 1082 AQKSGSMLVAQ 1083 AQKSQSLAGAQ 1084 AQKSQTSYMAQ 1085 AQKTTTSLAAQ 1086 AQLAGRGAGAQ 1087 AQLAPIMREAQ 1088 AQLATTRVEAQ 1089 AQLDRANPQAQ 1090 AQLEHRSGSAQ 1091 AQLFGPLLLAQ 1092 AQLFSWRNAAQ 1093 AQLFTTSNIAQ 1094 AQLGHSLTSAQ 1095 AQLGHSSQIAQ 1096 AQLGNLGHQAQ 1097 AQLGRVQSDAQ 1098 AQLGSSSVSAQ 1099 AQLHGPTLLAQ 1100 AQLHGVPMNAQ 1101 AQLHHNRESAQ 1102 AQLHNFPDNAQ 1103 AQLHSIGTSAQ 1104 AQLHSSTLGAQ 1105 AQLIHNGAAAQ 1106 AQLKVGPMDAQ 1107 AQLKVPVLMAQ 1108 AQLLDHISKAQ 1109 AQLLPATRDAQ 1110 AQLNHGANAAQ 1111 AQLNHTRPGAQ 1112 AQLNSPSFLAQ 1113 AQLNSSRASAQ 1114 AQLNSVKHYAQ 1115 AQLNTTKTNAQ 1116 AQLPGRSTSAQ 1117 AQLPSYHSAAQ 1118 AQLQRSTDGAQ 1119 AQLQTTYSSAQ 1120 AQLRNSSGDAQ 1121 AQLRSLSAEAQ 1122 AQLSHSYSSAQ 1123 AQLSNSVKTAQ 1124 AQLSSHAYQAQ 1125 AQLSSRPDAAQ 1126 AQLSSSRSAAQ 1127 AQLSSSYTVAQ 1128 AQLSTSYSSAQ 1129 AQLTHVSNIAQ 1130 AQLTTSVRIAQ 1131 AQLTTTRTEAQ 1132 AQLTVAGRSAQ 1133 AQMGSKSNEAQ 1134 AQMKVPILLAQ 1135 AQMLAGRESAQ 1136 AQMNAGRSGAQ 1137 AQMNSSKPTAQ 1138 AQMNSVRTGAQ 1139 AQMNTSRSPAQ 1140 AQMNTTRVPAQ 1141 AQMPTKMTDAQ 1142 AQMRTGSNEAQ 1143 AQMRTLTGDAQ 1144 AQMRTSHTEAQ 1145 AQMRTSNLTAQ 1146 AQMSGHRQDAQ 1147 AQMSHSSGAAQ 1148 AQMSNRVLEAQ 1149 AQMSTTRSSAQ 1150 AQMTSKPESAQ 1151 AQMVNRVQSAQ 1152 AQMVRSNDPAQ 1153 AQMYTTGQSAQ 1154 AQNAGRYGDAQ 1155 AQNGARTPPAQ 1156 AQNGQRLSPAQ 1157 AQNGRVGSNAQ 1158 AQNGSATRVAQ 1159 AQNGSKLAGAQ 1160 AQNGSRVIAAQ 1161 AQNGTMRLAAQ 1162 AQNHRPSGDAQ 1163 AQNLANTRDAQ 1164 AQNLQPGKIAQ 1165 AQNLRGTSVAQ 1166 AQNLSSVNGAQ 1167 AQNRTGGFDAQ 1168 AQNRVAQGEAQ 1169 AQNSATMRQAQ 1170 AQNSDPYRFAQ 1171 AQNSHISRDAQ 1172 AQNSQTLRSAQ 1173 AQNSRAAEHAQ 1174 AQNSRGPVAAQ 1175 AQNSSHANSAQ 1176 AQNSTQYNAAQ 1177 AQNTGHASMAQ 1178 AQNTGRSDSAQ 1179 AQNTNMPQYAQ 1180 AQNTSSVRGAQ 1181 AQNTVNVSGAQ 1182 AQNVAGRLDAQ 1183 AQNVSNLHGAQ 1184 AQNVSQLRDAQ 1185 AQNVTRGHEAQ 1186 AQNVYNVSGAQ 1187 AQPARPNNPAQ 1188 AQPFTPKPMAQ 1189 AQPGVGKQPAQ 1190 AQPHDSVMWAQ 1191 AQPINLPMSAQ 1192 AQPKSSHEWAQ 1193 AQPNNTIRGAQ 1194 AQPQKTGSAAQ 1195 AQPQSGPLPAQ 1196 AQPQSNRPAAQ 1197 AQPQSRGDSAQ 1198 AQPRLPEVFAQ 1199 AQPRTGTPPAQ 1200 AQPSHSVLFAQ 1201 AQPSRGQTAAQ 1202 AQPSRGQTTAQ 1203 AQPSTDTFWAQ 1204 AQPSVTGRSAQ 1205 AQPSYGGSGAQ 1206 AQPTGLPINAQ 1207 AQPTQRTGTAQ 1208 AQPTRGTGTAQ 1209 AQPTSALPMAQ 1210 AQPVSRSPAAQ 1211 AQQAGRSVAAQ 1212 AQQAGSSVAAQ 1213 AQQASRNEIAQ 1214 AQQATRGDSAQ 1215 AQQDRILSAAQ 1216 AQQDSMRGLAQ 1217 AQQDTRSHAAQ 1218 AQQGGLILLAQ 1219 AQQGSIRSDAQ 1220 AQQGYGGQTAQ 1221 AQQHAPSLLAQ 1222 AQQLPGGLHAQ 1223 AQQLSNKTNAQ 1224 AQQLSRDVPAQ 1225 AQQLVQHRDAQ 1226 AQQMRTGGIAQ 1227 AQQPGYSSDAQ 1228 AQQPRANQIAQ 1229 AQQPRTNESAQ 1230 AQQQLPVLMAQ 1231 AQQRESPLDAQ 1232 AQQRHLPTDAQ 1233 AQQSAGKLGAQ 1234 AQQSLSSLSAQ 1235 AQQSNLPMTAQ 1236 AQQSNVYREAQ 1237 AQQTSRLQEAQ 1238 AQQTTDRSGAQ 1239 AQQVGPILMAQ 1240 AQQVGSYQHAQ 1241 AQQVSTARPAQ 1242 AQQYGSVSQAQ 1243 AQQYSSGSSAQ 1244 AQRAGVSQIAQ 1245 AQRDTTYTTAQ 1246 AQRERSSEVAQ 1247 AQRGHGSSDAQ 1248 AQRGPLTLLAQ 1249 AQRGTLDLLAQ 1250 AQRIASPYDAQ 1251 AQRIGTATVAQ 1252 AQRIGTMSAAQ 1253 AQRLPGGLSAQ 1254 AQRLTQHEPAQ 1255 AQRMSNGSTAQ 1256 AQRMSSSSDAQ 1257 AQRNHSAGDAQ 1258 AQRNLPQVYAQ 1259 AQRNYSPTEAQ 1260 AQRPEVPNWAQ 1261 AQRPQGSAGAQ 1262 AQRPTMPEYAQ 1263 AQRSDRLTDAQ 1264 AQRSGMAVEAQ 1265 AQRSGSGSDAQ 1266 AQRSGSSADAQ 1267 AQRSNLLAEAQ 1268 AQRSNSSVGAQ 1269 AQRSTAIVAAQ 1270 AQRTGMTGAAQ 1271 AQRTGSVVNAQ 1272 AQRTGTQSSAQ 1273 AQRTIGTSTAQ 1274 AQRTSQIQSAQ 1275 AQRVGASGTAQ 1276 AQRVGSSLSAQ 1277 AQRVPAGGGAQ

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1294 AQSHEKFLYAQ 1295 AQSHSASREAQ 1296 AQSHSNTGNAQ 1297 AQSIPGKGMAQ
1298 AQSIRSQLPAQ 1299 AQSISAGRVAQ 1300 AQSISGRSDAQ 1301 AQSISGRVGAQ 1302
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AQSPRSNVTAQ 1315 AQSPRSSIGAQ 1316 AQSPVAPNWAQ 1317 AQSQALRADAQ 1318
AQSQGVRVPAQ 1319 AQSQKMLHGAQ 1320 AQSQPGPFNAQ 1321 AQSQSAKGPAQ 1322
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AQSRSQATDAQ 1327 AQSRSTDTGAQ 1328 AQSRVGLSTAQ 1329 AQSSAGKSPAQ 1330
AQSSAGRTGAQ 1331 AQSSATVREAQ 1332 AQSSDSRSSAQ 1333 AQSSGISRDAQ 1334
AQSSGPIILAQ 1335 AQSSHSANMAQ 1336 AQSSIVRQEAQ 1337 AQSSQNVSGAQ 1338
AQSSQRGHEAQ 1339 AQSSSSRGPAQ 1340 AQSSTVRNAAQ 1341 AQSSVTHSSAQ 1342
AQSSYQSNGAQ 1343 AQSTAARPVAQ 1344 AQSTGHNTQAQ 1345 AQSTGSSTSAQ 1346
AQSTGTPNWAQ 1347 AQSTHIARDAQ 1348 AQSTIVYREAQ 1349 AQSTMQNRIAQ 1350
AQSTNHGNNAQ 1351 AQSTQIHAGAQ 1352 AQSTQLRSGAQ 1353 AQSTRALQPAQ 1354
AQSTRSSDSAQ 1355 AQSTRTAPEAQ 1356 AQSTSNRPAAQ 1357 AQSTSPPRPAQ 1358
AQSTSRLPAAQ 1359 AQSTSSHTPAQ 1360 AQSTSSRTSAQ 1361 AQSTSSRTTAQ 1362
AQSTTSAPGAQ 1363 AQSTVTAVGAQ 1364 AQSVGELPFAQ 1365 AQSVPAKGSAQ 1366
AQSVRAGLNAQ 1367 AQSVRSPVTAQ 1368 AQSVRSSDYAQ 1369 AQSVTRTVAAQ 1370
AQSVYSHSSAQ 1371 AQSYAPQLLAQ 1372 AQSYSNRSVAQ 1373 AQTAAVRSGAQ 1374
AQTALKAVGAQ 1375 AQTANTRAEAQ 1376 AQTASQLSAAQ 1377 AQTDRITISAQ 1378
AQTDTRHATAQ 1379 AQTERNTASAQ 1380 AQTFHAVKEAQ 1381 AQTGSLAHGAQ 1382
AQTIGMRSAAQ 1383 AQTINKPNAAQ 1384 AQTISNSSGAQ 1385 AQTITRGTDAQ 1386
AQTLQNVNYAQ 1387 AQTLSSSREAQ 1388 AQTMGRNVSAQ 1389 AQTQGNRTDAQ 1390
AQTQHSTQSAQ 1391 AQTQSAYGSAQ 1392 AQTQSLRHDAQ 1393 AQTQTDRHSAQ 1394
AQTQTNIGRAQ 1395 AQTRHSSSDAQ 1396 AQTRSSSEAAQ 1397 AQTRSTIQEAQ 1398
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AQTSHVSSAAQ 1403 AQTSILRNEAQ 1404 AQTSNSSGQAQ 1405 AQTSNYGATAQ 1406
AQTSQSRVEAQ 1407 AQTSRANDTAQ 1408 AQTSRSNETAQ 1409 AQTSTPNKIAQ 1410
AQTSYSTTTAQ 1411 AQTTARNLEAQ 1412 AQTTGILRDAQ 1413 AQTTGTPRPAQ 1414
AQTTGYSQAAQ 1415 AQTTIRNAVAQ 1416 AQTTNITRTAQ 1417 AQTTSMGKVAQ 1418
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AQTVQSHSTAQ 1427 AQTVSSGRPAQ 1428 AQTYNATRSAQ 1429 AQVAGRNVSAQ 1430
AQVAGTSQGAQ 1431 AQVAGYSRDAQ 1432 AQVATPSFLAQ 1433 AQVFTNRSAAQ 1434
AQVGHGSLPAQ 1435 AQVGSYSRDAQ 1436 AQVGTLSSSAQ 1437 AQVGVNVHGAQ 1438
AQVIRGLEQAQ 1439 AQVISRDSSAQ 1440 AQVISSHHSAQ 1441 AQVKANQATAQ 1442
AQVLHASMSAQ 1443 AQVLHGSSYAQ 1444 AQVLRSNVMAQ 1445 AQVNLPVLLAQ 1446
AQVPGHSGLAQ 1447 AQVPGQSSLAQ 1448 AQVPRSSTDAQ 1449 AQVPRTGLTAQ 1450
AQVPTSHSTAQ 1451 AQVQSHGVNAQ 1452 AQVQYGGLTAQ 1453 AQVRGLYSDAQ 1454
AQVRHSGMEAQ 1455 AQVRPPNPSAQ 1456 AQVRSTLTDAQ 1457 AQVRSVGLDAQ 1458
AQVRTPLFAAQ 1459 AQVRTSYSDAQ 1460 AQVSGIRGDAQ 1461 AQVSGRSLSAQ 1462
AQVSNSYLGAQ 1463 AQVSNTYSGAQ 1464 AQVSRSESAAQ 1465 AQVSSYPNSAQ 1466
AQVTGLRGIAQ 1467 AQVTHASLSAQ 1468 AQVTQLGRSAQ 1469 AQVTRSAGEAQ 1470
AQVTSKNDLAQ 1471 AQVTTKTQLAQ 1472 AQVVANTSRAQ 1473 AQVVGSNREAQ 1474
AQVVRSGNEAQ 1475 AQVVRSVNDAQ 1476 AQVVRTPNSAQ 1477 AQWGSHPTGAQ

1478 AQWLKSVSSAQ 1479 AQWRHTSAEAQ 1480 AQYAGNSGNAQ 1481 AQYASSSNNAQ 1482 AQYERNHNAAQ 1483 AQYKSSLSEAQ 1484 AQYLHGLQQAQ 1485 AQYNQIRGDAQ 1486 AQYNSSNFGAQ 1487 AQYPGNRMEAQ 1488 AQYRNTVQDAQ 1489 AQYRQSSSDAQ 1490 AQYRSGTQEAQ 1491 AQYTGSISVAQ 1492 AQYTSSVTGAQ 1493 AQYVASSPMAQ 1494 AQYVGSGTAAQ 1495 AQYVHSNNAAQ 1496 AQYYSSSGGAQ

Description

DETAILED DESCRIPTION OF THE DISCLOSURE

[0013] In certain aspects the disclosure provides modified rAAVs with increased expression levels in the brain, heart, or brain and heart when compared to a parent AAV (e.g., AAV9).

[0014] In certain aspects, the disclosure provides rAAVs with a peptide insertion and or substitution comprising or consisting of an amino-acid sequence set forth in any one of Tables 1-3.

[0015] In certain embodiments, the parental AAV is AAV9. In some embodiments, the parental AAV comprises SEQ ID NO: 1. In various embodiments, the AAV capsid protein comprises a 7-mer insertion inserted into the parental AAV between amino acid 588 and amino acid 589 of the parent AAV, wherein positions 587-597 of the mutant AAV capsid protein (with AA position numbering including the insertion) are selected from the sequence provided in Tables 1-3 or selected from the group consisting of SEQ ID NO: 2-1496.

[0016] Generally, the insertion comprises a seven-amino acid sequence (7-mer) that is inserted or substituted at the 588 loop in a parental AAV capsid protein. Aspects provided herein provide amino acid insertions comprising seven amino acid polymer (7-mer) inserted at AA588-589 to produce an eleven amino acid polymer (11-mer) at the 588 loop of a parental AAV capsid protein.

[0017] Also disclosed herein are methods and kits for producing therapeutic recombinant AAV (rAAV) particles, as well as methods and pharmaceutical compositions or formulations comprising the rAAV particles, for the treatment of a disease or condition affecting the brain, heart, or brain and heart.

[0018] Disclosed herein are AAV capsids engineered with increased viral transduction in the brain, heart, or brain and heart. Disclosed further herein AAV capsids engineered with increased viral transduction in the heart. Advantageously, disclosed herein are AAV capsids engineered with increased viral transduction in the brain, heart, or brain and heart.

[0019] The AAV capsids can encapsidate a viral vector with a heterologous nucleic acid encoding, for example, a therapeutic gene expression product. Transduction of the heterologous nucleic acid in the brain, heart, or brain and heart can be achieved upon systemic delivery to a subject of the AAV capsid of the present disclosure encapsidating a heterologous nucleic acid. The AAV capsids disclosed herein are advantageous for many applications of gene therapy to treat human disease, including, but not limited to, disorders of the brain and/or heart.

[0020] The recombinant AAV vectors comprising a nucleic acid sequence encoding the AAV capsid proteins of the present disclosure as also provided herein. For example, the viral vectors of the present disclosure comprise a nucleic acid sequence comprising the AAV viral Cap (Capsid) encoding VP1, VP2, and VP3, at least one of which is modified to produce the AAV capsid proteins of the present disclosure. The recombinant AAV vector provided can be derived from an AAV serotype (e.g., AAV9) or a variant AAV serotype including an insertion of the present invention.

AAV Capsids

[0021] Provided herein are modified adeno-associated (AAV) virus capsid compositions useful for integrating a transgene into a target cell or environment (in a subject when they are administered systemically to the subject.

[0022] An rAAV comprises an AAV capsid that can be engineered to encapsidate a heterologous nucleic acid (e.g., therapeutic nucleic acid, gene editing machinery). The AAV capsid is made up of

three AAV capsid protein monomers, VP1, VP2, and VP3. Sixty copies of these three VP proteins interact in a 1:1:10 ratio to form the viral capsid. VP1 covers the whole of VP2 protein in addition to a ˜137 amino acid N-terminal region (VP1u), VP2 covers the whole of VP3 in addition to ˜65 amino acid N-terminal region (VP1/2 common region). The three capsid proteins share a conserved amino acid sequence of VP3, which in some cases is the region beginning at amino acid position 138 (e.g., AA139-736).

[0023] While not wishing to be bound by theory, it is understood that a parent AAV capsid sequence comprises a VP1 region. In certain embodiments, a parent AAV capsid sequence comprises a VP1, VP2 and/or VP3 region, or any combination thereof. A parent VP1 sequence may be considered synonymous with a parent AAV capsid sequence.

[0024] The AAV VP3 structure contains highly conserved regions that are common to all serotypes, a core eight-stranded β-barrel motif (βB-βI) and a small α-helix (αA). The loop regions inserted between the β-strands consist of the distinctive HI loop between β-strands H and I, the DE loop between β-strands D and E, and nine variable regions (VRs), which form the top of the loops. These VRs, such as the AA588 loop, are found on the capsid surface and can be associated with specific functional roles in the AAV life cycle including receptor binding, transduction and antigenic specificity.

[0025] In some aspects, the rAAV variant of the present invention comprises an AAV capsid protein having a peptide insertion at the residues corresponding to amino acids 588-589 of the AAV9 native sequence of SEQ ID NO: 1.

[0026] The AAV capsids comprise AAV capsid proteins (e.g., VP1, VP2, and VP3), each with an insertion, such as in the 588 loop of a parental AAV capsid protein structure (AAV9 VP1 numbering). The 588 loop contains the site of heparan sulfate binding of AAV2 and is amenable to peptide display. The only known receptors for AAV9 is N-linked terminal galactose and AAV receptor (AAVR), but many indications point toward there being others. Modifications to AAV9 588 loop are shown herein to confer an increased transgene transduction in target in vivo environments.

[0027] The present invention provides, in an aspect, a peptide insertion at the AAV 588 loop comprising or consisting of an amino-acid sequence set forth in any one of Tables 1-3.

[0028] Disclosed herein are AAV capsids comprising AAV capsid proteins with an insertion at the 588 loop that confer a higher transduction in brain cell types (e.g., brain endothelial cells, neurons, astrocytes) and/or heart cell types (e.g., cardiomyocytes, vascular cells, stromal cells, mesothelial cells). In particular, the AAV capsid proteins disclosed herein enable rAAV-mediated transduction of a heterologous nucleic acid (e.g., transgene) in the brain, heart, or brain and heart of a subject. The AAV capsids of the present disclosure may be formulated as a pharmaceutical composition. In addition, the AAV capsids can be isolated and purified to be used for a variety of applications.

[0029] In some embodiments, the rAAV capsid of the present disclosure are generated using the methods disclosed herein. In some instances, the rAAV capsid is chimeric. In some instances, the rAAV, or variant AAV protein comprises therein, confer an increase in a localization of the rAAV within the target tissue, as compared to the parental AAV capsid or capsid protein.

AAV Capsid Proteins

[0030] Disclosed herein are recombinant AAV (rAAV) capsids which comprise AAV capsid proteins that are engineered with a modified capsid protein (e.g., VP1, VP2, VP3). In some embodiments, the rAAV capsid proteins of the present disclosure are generated using the methods disclosed herein. In some embodiments, the AAV capsid proteins are used in the methods of delivering a therapeutic nucleic acid (e.g., a transgene) to a subject. In some instances, the rAAV capsid proteins have desired AAV expression rendering them particularly suitable for certain therapeutic applications, e.g., the treatment of a disease or disorder in a subject such as those disclosed herein.

[0031] The rAAV capsid proteins may be engineered for optimized expression in the CNS, for

example the brain, of a subject upon systemic administration of the rAAV to the subject. The rAAV capsid proteins are engineered to include the insertions provided in Tables 1-2. The rAAV capsid proteins including the insertions provided in Tables 1-2 are engineered to achieve efficient transduction of an encapsidated transgene. In particular, the rAAV capsid proteins have increased expression in the brain of a subject.

[0032] The rAAV capsid proteins may also be engineered for optimized expression in the heart of a subject upon systemic administration of the rAAV to the subject. The rAAV capsid proteins are engineered to include the insertions provided in Tables 2-3. The rAAV capsid proteins including the insertions provided in Tables 2-3 are engineered to achieve efficient transduction of an encapsidated transgene. In particular, the rAAV capsid proteins have increased expression in the heart of a subject.

[0033] The rAAV capsid proteins may also be engineered for optimized expression in the brain and heart of a subject upon systemic administration of the rAAV to the subject. The rAAV capsid proteins are engineered to include the insertions provided in Table 2. The rAAV capsid proteins including the insertions provided in Table 2 are engineered to achieve efficient transduction of an encapsidated transgene. In particular, the rAAV capsid proteins have increased expression in the brain and heart of a subject.

[0034] The engineered AAV capsid proteins described herein have, in some cases, an insertion of an amino acid that is heterologous to the parental AAV capsid protein at amino acid positions in the 588 loop. In some embodiments, the amino acid is not endogenous to the parental AAV capsid protein at the amino acid position of the insertion. The amino acid may be a naturally occurring amino acid in the same or equivalent amino acid position as the insertion of the substitution in a different AAV capsid protein.

[0035] The 7-mers described herein were advantageously generated using polymerase chain reaction (PCR) with degenerate primers, where each of the seven amino acids is encoded by a deoxyribose nucleic acid (DNA) sequence. This method of generating random 7-mer amino acid sequences enables sampling of the 207 (1.28 billion) possible 7mer amino acid combinations at the protein level.

[0036] The rAAV capsid proteins of the present disclosure comprise an insertion of an amino acid in an amino acid sequence of an AAV capsid protein. The AAV capsid, from which an engineered AAV capsid protein of the present disclosure is produced, is referred to as a “parental” AAV capsid. The complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-2 is provided in GenBank Accession No. NC_001401 and Srivastava et al., J. Virol., 45:555-564 (1983); the complete genome of AAV-3 is provided in GenBank Accession No. NC_1829; the complete genome of AAV-4 is provided in GenBank Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of AAV-6 is provided in GenBank Accession No. NC_00 1862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249, respectively; the AAV-9 genome is provided in Gao et al., J. Virol., 78:6381-6388 (2004); the AAV-10 genome is provided in Mol. Ther., 13 (1): 67-76 (2006); the AAV-11 genome is provided in Virology, 330(2): 375-383 (2004); portions of the AAV-12 genome are provided in Genbank Accession No. DQ813647; portions of the AAV-13 genome are provided in Genbank Accession No. EU285562.

[0037] In some cases, the parental AAV is derived from an AAV with a serotype selected from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11 and AAV12. The AAV capsid protein that is “derived” from another may be a variant AAV capsid protein. A variant may include, for example, a heterologous amino acid in an amino acid sequence of the AAV capsid protein. The heterologous amino acid may be non-naturally occurring in the AAV capsid protein. The heterologous amino acid may be naturally occurring in a different AAV capsid protein. In some instances, the parental AAV capsid is described in US Pat Publication 2020/0165576 and U.S. Pat. App. Ser. No. 62/832,826 and PCT/US20/20778; the content of each of which is

incorporated herein.
[0038] In some instances, the parental AAV is AAV9. In some instances, the amino acid sequence of the AAV9 capsid protein comprises SEQ ID NO: 1. The amino acid sequence of AAV9 VP1 capsid protein is provided in SEQ ID NO: 1
TABLE-US-00004
(MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLP
GYKYLGPGNGLDKGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHAD
AEFQERLKEDTSFGGNLGRAVFQAKKRLLEPLGLVEEAAKTAPGKKRPV
EQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTESVPDPQPIGEPPAAP
SGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVITTST
RTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHF
SPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTV
QVFTDSDYQLPYVLGSAHEGCLPPFPADVFMIPQYGYLTLNDGSQAVGR
SSFYCLEYFPSQMLRTGNNFQFSYEFENVPFHSSYAHSQSLDRLMNPLI
DQYLYYLSKTINGSGQNQQTLKFSVAGPSNMAVQGRNYIPGPSYRQQRV
STTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLS
GSLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSA
QAQAQTGWVQNQGILPGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGMKHPPPQILIKNTPVPADPPTAFNKDKLNSFITQYSTGQVSVEIEW
ELQKENSKRWNPEIQYTSNYYKSNNVEFAVNTEGVYSEPRPIGTRYLTR NL).
[0039] In some instances, the parental AAV capsid protein sequence is 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% homologous to SEQ ID NO: 1.
[0040] AAV capsid proteins from native AAV serotypes, such as AAV9, with tropisms including the liver activate the innate immune response, which in some cases causes a severe inflammatory response in a subject, which can lead to multi-organ failure. By improving transduction of a native AAV serotype for a target in vivo tissue (e.g., brain and/or heart) the rAAV particles of the present disclosure reduce the immunogenic properties of AAV-mediated transgene delivery and prevent activation of the innate immune response. Moreover, with increased transduction efficiency, the amount of the AAV composition needed to be delivered for effective transgene expression can be reduced. By reducing the total volume of the transgene delivered, off-target delivery and expression of the transgene is reduced.
[0041] In some instances, the parental AAV capsid protein comprises the entire VP1 region provided in SEQ ID NO: 1 (e.g., amino acids 1-736). In some instances, the parental AAV capsid protein comprises amino acids 217-736 in SEQ ID NO: 1, which is the common region found in VP1, VP2 and VP3 AAV9 capsid proteins. In some instances, the AAV capsid protein comprises amino acids 64-736 in SEQ ID NO: 1, which is the common region found in VP1 and VP2. The parental AAV capsid protein sequence may comprise amino acids selected from 1-736, 10-736, 20-736, 30-736, 40-736, 50-736, 60-736, 70-736, 80-736, 90-736, 100-736, 110-736, 120-736, 130-736, 140-736, 150-736, 160-736, 170-736, 180-736, 190-736, 200-736, 210-736, 220-736, 230-736, 240-736, 250-736, 260-736, 270-736, 280-736, 290-736, 300-736, 310-736, 320-736, 330-736, 340-736, 350-736, 360-736, 370-736, 380-736, 390-736, 400-736, 410-736, 420-736, 430-736, 440-736, and 450-736, from SEQ ID NO: 1. In some aspects, the rAAV variant comprises an AAV capsid protein comprising an amino acid sequence that is at least 98% identical to amino acid 217 to amino acid 736 of SEQ ID NO: 1. In some instances, the amino acid insertion is at a three (3)-fold axis of symmetry of a corresponding parental AAV capsid protein.
[0042] Disclosed herein are insertions of an amino acid sequence in an AAV capsid protein. Where the sequence numbering designation “588-589” is noted for AAV9, for example AAV VP1, the invention also includes insertions in similar locations in the other AAV serotypes. As used herein, “AA588-589” indicates that the insertion of the amino acid (or amino acid sequence) is immediately after an amino acid (AA) at position 588 and immediately before an AA at position

589 within an amino acid sequence of a parental AAV VP capsid protein (VP1 numbering). Amino acids 587-591 include a motif comprising “AQAQA” as set forth in SEQ ID NO: 1. It is envisioned that the sequences disclosed herein (Tables 1-3) may be inserted at AA588-589 in an amino acid sequence of a parental AAV9 capsid protein, a variant thereof, or equivalent amino acid position of a parental AAV of a different serotype (e.g., AAV1, AAV2, AAV3, and the like). In certain embodiments, the aforementioned “AQAQ” sequence flanking the insertion may include one or more substitutions.

[0043] The insertions described herein may, in some cases, comprise a 7-mer insertion at AA588-589.

[0044] Disclosed herein are AAV capsid proteins with an insertion described above in a parental AAV capsid protein that confers an increased transduction in the brain, heart, or brain and heart in a subject, even when delivered systemically. One of the many advantages of the AAV capsid proteins described herein is their ability to target tissue and cells within the brain, heart, or brain and heart. The tissue can be the brain, heart, or brain and heart.

[0045] Non-limiting examples of brain cells include a neuron and a glial cell. Glial cells can be selected from an oligodendrocyte, an ependymal cell, an astrocyte and a microglia.

[0046] Non-limiting examples of heart cells include cardiomyocytes, vascular cells, stromal cells, mesothelial cells

[0047] In some instances, the AAV capsid protein comprises an insertion/substitution of at least or about seven, eight, nine, ten or eleven amino acids of an amino acid sequence of Tables 1-3 at an amino acid position 588-589 in a parental AAV9 capsid protein (SEQ ID NO: 1).

[0048] In some cases, the AAV capsid protein has an increased viral transduction enrichment in brain, heart, or brain and heart.

[0049] The rAAV capsid proteins described herein may be isolated and purified. The AAV may be isolated and purified by methods standard in the art such as by column chromatography, iodixanol gradients, or cesium chloride gradients. Methods for purifying AAV from helper virus are known in the art and may include methods disclosed in, for example, Clark et al., Hum. Gene Ther., 10 (6): 1031-1039 (1999); Schenpp and Clark, Methods Mol. Med., 69:427-443 (2002); U.S. Pat. No. 6,566,118 and WO 98/09657.

[0050] In addition, the AAV capsid proteins disclosed herein, either isolated and purified, or not, may be formulated into a pharmaceutical formulation, which in some cases, further comprises a pharmaceutically acceptable carrier.

[0051] The rAAV capsid protein can be conjugated to a nanoparticle, a second molecule, or a viral capsid protein. In some cases, the nanoparticle or viral capsid protein would encapsidate the therapeutic nucleic acid described herein. In some instances, the second molecule is a therapeutic agent, e.g., a small molecule, antibody, antigen-binding fragment, peptide, or protein, such as those described herein.

[0052] “Percent Identity” is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the Wisconsin Genetics Software Package is BLOSUM62 (see: Henikoff and Henikoff, (1989) Proc. Natl. Acad. Sci. USA 89:10915).

[0053] Sequence identity/similarity values provided herein can refer to the value obtained using the BLAST+ 2.5.0 suite of programs using default settings (blast.ncbi.nlm.nih.gov) (Camacho, C., et al. (2009) BLAST+: architecture and applications. BMC Bioinformatics 10:421).

[0054] As those of ordinary skill in the art will understand, BLAST searches assume that proteins can be modeled as random sequences. However, many real proteins comprise regions of nonrandom sequences, which may be homopolymeric tracts, short-period repeats, or regions enriched in one or more amino acids. Such low-complexity regions may be aligned between

unrelated proteins even though other regions of the protein are entirely dissimilar. A number of low-complexity filter programs can be employed to reduce such low-complexity alignments. For example, the SEG (Wooten and Federhen, (1993) Comput. Chem. 17:149-63) and XNU (Ci-ayerie and States (1993) Comput. Chem. 17:191-201) low-complexity filters can be employed alone or in combination.

[0055] The terms “substantial identity” and “substantially identical” indicate that a polypeptide or nucleic acid comprises a sequence with between 55-100% sequence identity to a reference sequence, with at least 55% sequence identity, or at least 60%, or at least 65%, or at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90%, or at least 95%, or at least 99% sequence identity or any percentage of value within the range of 55-100% sequence identity relative to the reference sequence. The percent sequence identity may occur over a specified comparison window. Optimal alignment may be ascertained or conducted using the homology alignment algorithm of Needleman and Wunsch, supra.

[0056] For example, the insertion sequences may include, but are not limited to, sequences that are not exactly the same as the sequences disclosed herein, but which have, in addition to the substitutions explicitly described for various sequences listed herein, additional substitutions of amino acid residues which substantially do not impair the activity or properties of the sequences described herein, such as those predicted by homology software e.g. BLOSUM62 matrices.

AAV Particles

[0057] The rAAV particles with the insertion sequences described herein have an increased transduction enrichment in the brain, heart, or brain and heart. In some instances, the increased transduction enrichment comprises a 1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold or 10-fold increase, or more. In some instances, the increased transduction enrichment is at least 1-fold. In some instances, the increased transduction enrichment is at least 2-fold. In some instances, the increased transduction enrichment is at least 4-fold.

[0058] The rAAV particles with the insertion sequences described herein have an increased expression enrichment in the brain. Detecting whether a rAAV possesses more or less specificity for a target in vivo environment, includes measuring a level of gene expression product (e.g., RNA or protein) expressed from the heterologous nucleic acid encapsidated by the rAAV in a tissue sample obtained from a subject. Suitable methods for measuring expression of a gene expression product include next-generation sequencing (NGS) and quantitative polymerase chain reaction (qPCR).

[0059] The increased expression in the brain, heart, or brain and heart is represented by the enrichment values provided in Tables 1-3

Heterologous Nucleic Acids

[0060] Disclosed herein are therapeutic nucleic acids useful for the treatment or prevention of a disease or condition, or symptom of the disease or condition. In some embodiments, the therapeutic nucleic acids encode a therapeutic gene expression product. Non-limiting examples of gene expression products include proteins, polypeptides, peptides, enzymes, antibodies, antigen binding fragments, nucleic acid (RNA, DNA, antisense oligonucleotide, siRNA, and the like), and gene editing components, for use in the treatment, prophylaxis, and/or amelioration of the disease or disorder, or symptoms of the disease or disorder. In some instances, the therapeutic nucleic acids are placed in an organism, cell, tissue or organ of a subject by way of a rAAV, such as those disclosed herein.

[0061] Disclosed herein are rAAVs, each comprising a viral vector (e.g., a single stranded DNA molecule (ssDNA)). In some instances, the viral vector comprises two inverted terminal repeat (ITR) sequences that are about 145 bases each, flanking a transgene. In some embodiments, the transgene comprises a therapeutic nucleic acid, and in some cases, a promoter in cis with the therapeutic nucleic acid in an open reading frame (ORF). The promoter is capable of initiating transcription of therapeutic nucleic acid in the nucleus of the target cell. The ITR sequences can be

from any AAV serotype. Non-limiting examples of AAV serotypes include AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and AAV12. In some cases, an ITR is from AAV2. In some cases, an ITR is from AAV9.

[0062] Disclosed herein are transgenes that can comprise any number of nucleotides. In some cases, a transgene can comprise less than about 100 nucleotides. In some cases, a transgene can comprise at least about 100 nucleotides. In some cases, a transgene can comprise at least about 200 nucleotides. In some cases, a transgene can comprise at least about 300 nucleotides. In some cases, a transgene can comprise at least about 400 nucleotides. In some cases, a transgene can comprise at least about 500 nucleotides. In some cases, a transgene can comprise at least about 1000 nucleotides. In some cases, a transgene can comprise at least about 5000 nucleotides. In some cases, a transgene can comprise over 5,000 nucleotides. In some cases, a transgene can comprise between about 500 and about 5000 nucleotides. In some cases, a transgene comprises about 5000 nucleotides. In any of the cases disclosed herein, the transgene can comprise DNA, RNA, or a hybrid of DNA and RNA. In some cases, the transgene can be single stranded. In some cases, the transgene can be double stranded.

[0063] Disclosed herein are transgenes useful for modulating the expression or activity of a target gene or gene expression product thereof. In some instances, the transgene is encapsidated by an rAAV capsid protein of an rAAV particle described herein. In some instances, the rAAV particle is delivered to a subject to treat a disease or condition disclosed herein in the subject. In some instances, the delivery is systemic.

[0064] The transgenes disclosed herein are useful for expressing an endogenous gene at a level similar to that of a healthy or normal individual. This is particularly useful in the treatment of a disease or condition related to the underexpression, or lack of expression, of a gene expression product. In some embodiments, the transgenes disclosed herein are useful for overexpressing an endogenous gene, such that an expression level of the endogenous gene is above the expression level of a healthy or normal individual. Additionally, transgenes can be used to express exogenous genes (e.g., active agent such as an antibody, peptide, nucleic acid, or gene editing components). In some embodiments, the therapeutic gene expression product is capable of altering, enhancing, increasing, or inducing the activity of one or more endogenous biological processes in the cell. In some embodiments, the transgenes disclosed herein are useful for reducing expression of an endogenous gene, for example, a dominant negative gene. In some embodiments, the therapeutic gene expression product is capable of altering, inhibiting, reducing, preventing, eliminating, or impairing the activity of one or more endogenous biological processes in the cell. In some aspects, the increase of gene expression refers to an increase by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%. In certain aspects, the protein product of the targeted gene may be increased by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%. In some aspects, the decrease of gene expression refers to an increase by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%. In certain aspects, the protein product of the targeted gene may be decreased by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%.

[0065] When endogenous sequences (endogenous or part of a transgene) are expressed with a transgene, the endogenous sequences can be full-length sequences (wild-type or mutant) or partial sequences. The endogenous sequences can be functional. Non-limiting examples of the function of these full length or partial sequences include increasing the serum half-life of the polypeptide expressed by a transgene (e.g., therapeutic gene) and/or acting as a carrier.

[0066] A transgene can be inserted into an endogenous gene such that all, some or none of the endogenous gene is expressed. For example, a transgene as described herein can be inserted into an endogenous locus such that some (N-terminal and/or C-terminal to a transgene) or none of the endogenous sequences are expressed, for example as a fusion with a transgene. In other cases, a transgene (e.g., with or without additional coding sequences of the endogenous gene) is integrated

into any endogenous locus, for example a safe-harbor locus. For example, a Frataxin (FXN) transgene can be inserted into an endogenous FXN gene. A transgene can be inserted into any gene, e.g., the genes as described herein.

[0067] At least one advantage of the present disclosure is that virtually any therapeutic nucleic acid may be used to express any therapeutic gene expression product. In some instances, the therapeutic gene expression product is a therapeutic protein or a peptide (e.g., antibody, antigen-binding fragment, peptide, or protein). In one embodiment the protein encoded by the therapeutic nucleic acid is between 50-5000 amino acids in length. In some embodiments the protein encoded is between 50-2000 amino acids in length. In some embodiments the protein encoded is between 50-1000 amino acids in length. In some embodiments the protein encoded is between 50-1500 amino acids in length. In some embodiments the protein encoded is between 50-800 amino acids in length. In some embodiments the protein encoded is between 50-600 amino acids in length. In some embodiments the protein encoded is between 50-400 amino acids in length. In some embodiments the protein encoded is between 50-200 amino acids in length. In some embodiments the protein encoded is between 50-100 amino acids in length. In some embodiments the peptide encoded is between 4-50 amino acids in length. In some embodiments, the protein encoded is a tetrapeptide, a pentapeptide, a hexapeptide, a heptapeptide, an octapeptide, a nonapeptide, or a decapeptide. In some embodiments, the protein encoded comprises a peptide of 2-30 amino acids, such as for example 5-30, 10-30, 2-25, 5-25, 10-25, or 10-20 amino acids. In some embodiments, the protein encoded comprises a peptide of at least 11, 12, 13, 14, 15, 17, 20, 25 or 30 amino acids, or a peptide that is no longer than 50 amino acids, e.g. no longer than 35, 30, 25, 20, 17, 15, 14, 13, 12, 11 or 10 amino acids.

[0068] Non-limiting examples of therapeutic protein or peptides include an adrenergic agonist, an anti-apoptosis factor, an apoptosis inhibitor, a cytokine receptor, a cytokine, a cytotoxin, an erythropoietic agent, a glutamic acid decarboxylase, a glycoprotein, a growth factor, a growth factor receptor, a hormone, a hormone receptor, an interferon, an interleukin, an interleukin receptor, a kinase, a kinase inhibitor, a nerve growth factor, a netrin, a neuroactive peptide, a neuroactive peptide receptor, a neurogenic factor, a neurogenic factor receptor, a neuropilin, a neurotrophic factor, a neurotrophin, a neurotrophin receptor, an N-methyl-D-aspartate antagonist, a plexin, a protease, a protease inhibitor, a protein decarboxylase, a protein kinase, a protein kinsase inhibitor, a proteolytic protein, a proteolytic protein inhibitor, a semaphoring, a semaphorin receptor, a serotonin transport protein, a serotonin uptake inhibitor, a serotonin receptor, a serpin, a serpin receptor, and a tumor suppressor. In certain embodiments, the therapeutic protein or peptide is selected from brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), macrophage colony-stimulating factor (CSF), epidermal growth factor (EGF), fibroblast growth factor (FGF), gonadotropin, interferon-gamma (IFN), insulin-like growth factor 1 (IFG-1), nerve growth factor (NGF), platelet-derived growth factor (PDGF), pigment epithelium-derived factor (PEDF), transforming growth factor (TGF), transforming growth factor-beta (TGF-B), tumor necrosis factor (TNF), vascular endothelial growth factor (VEGF), prolactin, somatotropin, X-linked inhibitor of apoptosis protein 1 (XIAP1), interleukin 1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-10, viral IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, and IL-18.

[0069] A therapeutic gene expression product can comprise gene editing components. Non-limiting examples of gene editing components include those required for CRISPR/Cas, artificial site-specific RNA endonuclease (ASRE), zinc finger endonuclease (ZFN), and transcription factor like effector nuclease (TALEN). In a non-limiting example, a subject having Huntington's disease is identified. The subject is then systemically administered a first amount of a rAAV encapsidating a viral vector encoding ZFN engineered to represses the transcription of the Huntingtin (HTT) gene. The rAAV will include a modified AAV capsid protein that includes an amino acid sequence provided in any one of Tables 1-3, so as to allow proper targeting of the ZFN to the brain and/or

heart system. If needed, the subject is administered a second or third dose of the rAAV, until a therapeutically effective amount of the ZFN is expressed in the subject's nervous system.

[0070] A therapeutic nucleic acid can comprise a non-protein coding gene e.g., sequences encoding antisense RNAs, RNAi, shRNAs and micro RNAs (miRNAs), miRNA sponges or decoys, recombinase delivery for conditional gene deletion, conditional (recombinase-dependent) expression, includes those required for the gene editing components described herein. The non-protein coding gene may also encode a tRNA, rRNA, tmRNA, piRNA, double stranded RNA, snRNA, snoRNA, and/or long non-coding RNA (lncRNA). In some cases, the non-protein coding gene can modulate the expression or the activity of a target gene or gene expression product. For example, the RNAs described herein may be used to inhibit gene expression in the brain, heart, or brain and heart. In some cases, inhibition of gene expression refers to an inhibition by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%. In some cases, the protein product of the targeted gene may be inhibited by at least about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95% and 100%. The gene can be either a wild type gene or a gene with at least one mutation. The targeted protein may be either a wild type protein or a protein with at least one mutation.

[0071] A therapeutic nucleic acid can modulate the expression or activity of a gene or gene expression product expressed from the gene that is implicated in a disease or disorder of the brain, heart, or brain and heart. For example, the therapeutic nucleic acid, in some cases is a gene or a modified version of the gene described herein. In some instances, the gene or gene expression product is inhibited. In some instances, the gene or gene expression product is enhanced.

[0072] In another example, the therapeutic nucleic acid comprises an effector gene expression product such as a gene editing component specific to target a gene therein. Non-limited examples of genes include target gene or gene expression product selected from ATP1A2, CACNAIA, SETD5, SHANK3, NF2, DNMT1, TCF4, RAI1, PEX1, ARSA, EIF2B5, EIF2B1, EIF2B2, NPC1, ADAR, MFSD8, STXBP1, PRICKLE2, PRRT2, IDUA, STX1B, Sarcoglycan Alpha (SGCA), glutamic acid decarboxylase 65 (GAD65), glutamic acid decarboxylase 67 (GAD67), CLN2, Nerve Growth Factor (NGF), glial cell derived neurotrophic factor (GDNF), Survival Of Motor Neuron 1, STXBP1, Telomeric (SMN1), Factor X (FIX), Retinoid Isomerohydrolase (RPE65), sarco/endoplasmic reticulum Ca2+-ATPase (SERCA2a), Glucocerebrosidase (GCase), galactocerebrosidase (GALC), CDKL5, Frataxin (FXN), Huntingtin (HTT), methyl-CpG binding protein 2 (MECP2), a peroxisomal biogenesis factor (PEX), progranulin (GRN), an antitubulin agent, copper-zinc superoxide dismutase (SOD1), iduronate 2 sulfatase (hIDS), Glucosylceramidase Beta (GBA), fragile X mental retardation 1 (FMR1), NPC Intracellular Cholesterol Transporter 1 (NPC1), SCN1A, C9orf72, NPS3 and a NLRP3 inflammasome. In some embodiments, the peroxisomal biogenesis factor (PEX) is selected from PEX1, PEX2, PEX3, PEX4, PEX5, PEX6, PEX7, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, and PEX26. In some instances, the gene or gene expression product is inhibited. In some instances, the gene or gene expression product is enhanced.

AAV Vectors

[0073] Aspects disclosed herein comprise plasmid vectors comprising a nucleic acid sequence encoding the AAV capsids and AAV capsid proteins described herein. AAV vectors described herein are useful for the assembly of a rAAV and viral packaging of a heterologous nucleic acid. In addition, an AAV vector may encode a transgene comprising the heterologous nucleic acid.

[0074] An AAV vector can comprise a transgene, which in some cases encodes a heterologous gene expression product (e.g., therapeutic gene expression product, recombinant capsid protein, and the like). The transgene is in cis with two inverted terminal repeats (ITRs) flanking the transgene. The transgene may comprise a therapeutic nucleic acid encoding a therapeutic gene expression product. Due to the limited packaging capacity of the rAAV (˜5 KB), in some cases, a longer transgene may be split between two AAV vectors, the first with 3′ splice donor and the second with a 5′ splice

acceptor. Upon co-infection of a cell, concatemers form, which are spliced together to express a full-length transgene.

[0075] A transgene is generally inserted so that its expression is driven by the endogenous promoter at the integration site, namely the promoter that drives expression of the endogenous gene into which a transgene is inserted. In some instances, a transgene comprises a promoter and/or enhancer, for example a constitutive promoter or an inducible or tissue/cell specific promoter. As a non-limiting example, the promoter may be CMV promoter, a CMV-β-Actin-intron-β-Globin hybrid promoter (CAG), CBA promoter, FRDA or FXN promoter, UBC promoter, GUSB promoter, NSE promoter, Synapsin promoter, MeCP2 promoter, GFAP promoter, H1 promoter, U6 promoter, NFL promoter, NFH promoter, SCN8A promoter, or PGK promoter. As a non-limiting example, promoters can be tissue-specific expression elements include, but are not limited to, human elongation factor 1α-subunit (EF1α), immediate-early cytomegalovirus (CMV), chicken β-actin (CBA) and its derivative CAG, the β glucuronidase (GUSB), and ubiquitin C (UBC). The transgene may include a tissue-specific expression elements for neurons such as, but not limited to, neuron-specific enolase (NSE), platelet-derived growth factor (PDGF), platelet-derived growth factor B-chain (PDGF-β), the synapsin (Syn), the methyl-CpG binding protein 2 (MeCP2), Ca2+/calmodulin-dependent protein kinase II (CaMKII), metabotropic glutamate receptor 2 (mGluR2), NFL, NFH, np32, PPE, Enk and EAAT2 promoters. The transgene may comprise a tissue-specific expression element for astrocytes such as, but not limited to, the glial fibrillary acidic protein (GFAP) and EAAT2 promoters. The transgene may comprise tissue-specific expression elements for oligodendrocytes such as, but not limited to, the myelin basic protein (MBP) promoter.

[0076] In some embodiments, the promoter is less than 1 kb. The promoter may have a length of 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 450, 460, 470, 480, 490, 500, 510, 520, 530, 540, 550, 560, 570, 580, 590, 600, 610, 620, 630, 640, 650, 660, 670, 680, 690, 700, 710, 720, 730, 740, 750, 760, 770, 780, 790, 800 or more than 800. The promoter may have a length between 200-300, 200-400, 200-500, 200-600, 200-700, 200-800, 300-400, 300-500, 300-600, 300-700, 300-800, 400-500, 400-600, 400-700, 400-800, 500-600, 500-700, 500-800, 600-700, 600-800 or 700-800. The promoter may provide expression of the therapeutic gene expression product for a period of time in targeted tissues such as, but not limited to, the brain, heart, or brain and heart. Expression of the therapeutic gene expression product may be for a period of 1 hour, 2, hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, 16 hours, 17 hours, 18 hours, 19 hours, 20 hours, 21 hours, 22 hours, 23 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 8 days, 9 days, 10 days, 11 days, 12 days, 13 days, 2 weeks, 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 3 weeks, 22 days, 23 days, 24 days, 25 days, 26 days, 27 days, 28 days, 29 days, 30 days, 31 days, 1 month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 1 year, 13 months, 14 months, 15 months, 16 months, 17 months, 18 months, 19 months, 20 months, 21 months, 22 months, 23 months, 2 years, 3 years, 4 years, 5 years, 6 years, 7 years, 8 years, 9 years, 10 years, 11 years, 12 years, 13 years, 14 years, 15 years, 16 years, 17 years, 18 years, 19 years, 20 years, 21 years, 22 years, 23 years, 24 years, 25 years, 26 years, 27 years, 28 years, 29 years, 30 years, 31 years, 32 years, 33 years, 34 years, 35 years, 36 years, 37 years, 38 years, 39 years, 40 years, 41 years, 42 years, 43 years, 44 years, 45 years, 46 years, 47 years, 48 years, 49 years, 50 years, 55 years, 60 years, 65 years, or more than 65 years. Expression of the payload may be for 1-5 hours, 1-12 hours, 1-2 days, 1-5 days, 1-2 weeks, 1-3 weeks, 1-4 weeks, 1-2 months, 1-4 months, 1-6 months, 2-6 months, 3-6 months, 3-9 months, 4-8 months, 6-12 months, 1-2 years, 1-5 years, 2-5 years, 3-6 years, 3-8 years, 4-8 years or 5-10 years or 10-15 years, or 15-20 years, or 20-25 years, or 25-30 years, or 30-35 years, or 35-40 years, or 40-45 years, or 45-50 years, or 50-55 years, or 55-60 years, or 60-65 years.

[0077] An AAV vector can comprise a genome of a helper virus. Helper virus proteins are required for the assembly of a recombinant AAV (rAAV), and packaging of a transgene containing a heterologous nucleic acid into the rAAV. The helper virus genes are adenovirus genes E4, E2a and VA, that when expressed in the cell, assist with AAV replication. In some embodiments, an AAV vector comprises E2. In some embodiments, an AAV vector comprises E4. In some embodiments, an AAV vector comprises VA. In some instances, the AAV vector comprises one of helper virus proteins, or any combination.

[0078] The target gene or gene expression product for use in a transgene can be selected from ATP1A2, CACNAIA, SETD5, SHANK3, NF2, DNMT1, TCF4, RAI1, PEX1, ARSA, EIF2B5, EIF2B1, EIF2B2, NPC1, ADAR, MFSD8, STXBP1, PRICKLE2, PRRT2, IDUA, STX1B, Sarcoglycan Alpha (SGCA), glutamic acid decarboxylase 65 (GAD65), glutamic acid decarboxylase 67 (GAD67), CLN2, Nerve Growth Factor (NGF), glial cell derived neurotrophic factor (GDNF), Survival Of Motor Neuron 1, STXBP1, Telomeric (SMN1), Factor X (FIX), Retinoid Isomerohydrolase (RPE65), sarco/endoplasmic reticulum Ca2+-ATPase (SERCA2a), Glucocerebrosidase (GCase), galactocerebrosidase (GALC), CDKL5, Frataxin (FXN), Huntingtin (HTT), methyl-CpG binding protein 2 (MECP2), a peroxisomal biogenesis factor (PEX), progranulin (GRN), an antitubulin agent, copper-zinc superoxide dismutase (SOD1), iduronate 2 sulfatase (hIDS), Glucosylceramidase Beta (GBA), fragile X mental retardation 1 (FMR1), NPC Intracellular Cholesterol Transporter 1 (NPC1), SCN1A, C9orf72, NPS3 and a NLRP3 inflammasome. In some embodiments, the peroxisomal biogenesis factor (PEX) is selected from PEX1, PEX2, PEX3, PEX4, PEX5, PEX6, PEX7, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, and PEX26.

[0079] An AAV vector can comprise a viral genome comprising a nucleic acid encoding the recombinant AAV (rAAV) capsid protein described herein. The viral genome can comprise a Replication (Rep) gene encoding a Rep protein, and Capsid (Cap) gene encoding an AAP protein in the first open reading frame (ORF1) or a Cap protein in the second open reading frame (ORF2). The Rep protein is selected from Rep78, Rep68, Rep52, and Rep40. In some instances, the Cap gene is modified encoding a modified AAV capsid protein described herein. A wild-type Cap gene encodes three proteins, VP1, VP2, and VP3. In some cases, VP1 is modified. In some cases, VP2 is modified. In some cases, VP3 is modified. In some cases, all three VP1-VP3 are modified. The AAV vector can comprise nucleic acids encoding wild-type Rep78, Rep68, Rep52, Rep40 and AAP proteins.

Methods of Producing rAAVs

[0080] Disclosed herein are methods of producing the AAV capsids comprising the AAV capsid proteins and viral vector encoding a therapeutic nucleic acid. The AAV capsid proteins are produced by introducing into a cell (e.g., immortalized stem cell) a first vector containing a transgene cassette flanked by inverted terminal repeat (ITR) sequences from a parental AAV virus (the transgene cassette has a promoter sequence that drives transcription of a heterologous nucleic acid in the nucleus of the target cell), a second vector encoding the AAV genome with a AAV capsid protein (encoding the AAV Rep gene as well as the modified Cap gene for the variant being produced), and a third vector encoding helper virus proteins, required for assembly of the AAV capsid structure and packaging of the transgene in the modified AAV capsid structure. The assembled AAV capsid can be isolated and purified from the cell using suitable methods known in the art.

[0081] The transgenes contained in a recombinant AAV (rAAV) vector and encapsidated by the AAV capsid proteins of the present disclosure are also provided herein. The transgenes disclosed herein are delivered to a subject for a variety of purposes, such as to treat a disease or condition in the subject. The transgene can be gene editing components that modulate the activity or expression of a target gene or gene expression product. Alternatively, the transgene is a gene encoding a therapeutic gene expression product that is effective to modulate the activity or expression of itself,

or another target gene or gene expression product.
[0082] Aspects disclosed herein provide methods of manufacturing rAAV virus or virus particles comprising: (a) introducing into a cell a nucleic acid comprising: (i) first vector containing a transgene cassette flanked by inverted terminal repeat (ITR) sequences from a parental AAV virus (the transgene cassette has a promoter sequence that drives transcription of a heterologous nucleic acid in the nucleus of the target cell); (ii) a second vector encoding the AAV genome with a AAV capsid protein of the present invention; and (iii) a vector encoding helper virus proteins, required for assembly of the AAV capsid structure and packaging of the transgene in the modified AAV capsid structure; (b) expressing in the cell the AAV capsid protein described herein; (c) assembling an AAV particle comprising the AAV capsid proteins disclosed herein; and (d) packaging the AAV particle. In some instances, the cell is mammalian. In some instances, the cell is immortalized. In some instances, the immortalized cell is an embryonic stem cell. In some instances, the embryonic stem cell is a human embryonic stem cell. In some instances, the human embryonic stem cell is a human embryonic kidney 293 (HEK-293) cell. In some instances, the Cap gene is derived from the deoxyribose nucleic acid (DNA) provided in SEQ ID NO: 6. In some instances, the 5′ ITR and the 3′ ITR are derived from an AAV2 serotype. In some instances, the 5′ ITR and the 3′ ITR are derived from an AAV5 serotype. In some instances, the 5′ ITR and the 3′ ITR are derived from an AAV9 serotype. In some instances, the first nucleic acid sequence and the second nucleic acid sequence are in trans. In some instances, the first nucleic acid sequence and the second nucleic acid sequence are in cis. In some instances, the first nucleic acid sequence, the second nucleic acid sequence and the third nucleic acid sequence, are in trans.
[0083] The Cap gene disclosed here comprises any one of SEQ ID NOS: 57-73 from Table 4, which are DNA sequences encoding the modified AAV capsid protein portions of the present disclosure.
[0084] In some instances, the methods comprise packing the first nucleic acid sequence encoding the therapeutic gene expression product such that it becomes encapsidated by the modified AAV capsid protein. In some embodiments, the rAAV particles are isolated, concentrated, and purified using suitable viral purification methods, such as those described herein.
[0085] In some cases, rAAVs of the present disclosure are generated using the methods described in Challis, R. C. et al. Nat. Protoc. 14, 379 (2019). Briefly, triple transfection of HEK293T cells (ATCC) using polyethylenimine (PEI) is performed, viruses are collected after 120 hours from both cell lysates and media and purified over iodixanol. In a non-limiting example, the rAAVs are generated by triple transfection of precursor cells (e.g., HEK293T) cells using a standard transfection protocol (e.g., PEI). Viral particles are harvested from the media after a period of time (e.g., 72 h post transfection) and from the cells and media at a later point in time (e.g., 120 h post transfection). Virus present in the media is concentrated by precipitation with 8% polyethylene glycol (PEG) and 500 mM sodium chloride and the precipitated virus is added to the lysates prepared from the collected cells. The viruses are purified over iodixanol (Optiprep, Sigma) step gradients (15%, 25%, 40% and 60%). Viruses are concentrated and formulated in PBS. Virus titers are determined by measuring the number of DNaseI-resistant vector genome copies (VGs) using qPCR and the linearized genome plasmid as a control.
[0086] The cell can be selected from a human, a primate, a murine, a feline, a canine, a porcine, an ovine, a bovine, an equine, an epine, a caprine and a lupine host cell. In some instances, the cell is a progenitor or precursor cell, such as a stem cell. In some instances, the stem cell is a mesenchymal cell, embryonic stem cell, induced pluripotent stem cell (iPSC), fibroblast or other tissue specific stem cell. The cell can be immortalized. In some cases, the immortalized cell is a HEK293cell. In some instances, the cell is a differentiated cell. Based on the disclosure provided, it is expected that this system can be used in conjunction with any transgenic line expressing a recombinase in the target cell type of interest to develop AAV capsids that more efficiently transduce that target cell population.

Methods of Treatment

[0087] Disclosed herein are methods of treating a disease or condition, or a symptom of the disease or condition, in a subject, comprising administrating of therapeutically effective amount of one or more compositions (e.g., rAAV particle, AAV vector, pharmaceutical composition) disclosed herein to the subject. In some embodiments, the composition is a rAAV capsid protein described herein. In some embodiments, the composition is an isolated and purified rAAV capsid protein described herein. In some embodiments, the rAAV particle encapsidates an AAV vector comprising a transgene (e.g., therapeutic nucleic acid). In some embodiments, the composition is a rAAV capsid protein described herein conjugated with a therapeutic agent disclosed herein. In some embodiments, the composition is a pharmaceutical composition comprising the rAAV particle and a pharmaceutically acceptable carrier. In some embodiments, the one or more compositions are administered to the subject alone (e.g., stand-alone therapy). In some embodiments, the composition is a first-line therapy for the disease or condition. In some embodiments, the composition is a second-line, third-line, or fourth-line therapy, for the disease or condition.

[0088] Recombinant adeno-associated virus (rAAV) mediated gene delivery leverages the AAV mechanism of viral transduction for nuclear expression of an episomal heterologous nucleic acid (e.g., a transgene, therapeutic nucleic acid). Upon delivery to a host in vivo environment, a rAAV will (1) bind or attach to cellular surface receptors on the target cell, (2) endocytose, (3) traffic to the nucleus, (4) uncoat the virus to release the encapsidated heterologous nucleic acid, (5) convert of the heterologous nucleic acid from single-stranded to double-stranded DNA as a template for transcription in the nucleus, and (6) transcribe of the episomal heterologous nucleic acid in the nucleus of the host cell (“transduction”). rAAVs engineered to have an increased transduction enrichment (transcription of the episomal heterologous nucleic acid in the host cell) are desirable for gene therapy applications.

[0089] Aspects disclosed herein provide methods of treating a disease or condition in a subject, the method comprising administering to the subject a therapeutically effective amount of the rAAV of the present disclosure, or the pharmaceutical formulation of the present disclosure, wherein the gene product is a therapeutic gene product. In some embodiments, the administering is by intracranial, intraventricular, intracerebroventricular, intravenous, intraarterial, intranasal, intrathecal, intracisternae magna, or subcutaneous.

[0090] Provided here, are methods of treating a disease or a condition associated with an aberrant expression or activity of a target gene or gene expression product thereof, the method comprising modulating the expression or the activity of a target gene or gene expression product in a subject by administering a rAAV encapsidating a heterologous nucleic acid of the present disclosure. In some instances, the expression or the activity of the target gene or gene expression product is decreased, relative to that in a normal (non-diseased) individual; and administering the rAAV to the subject is sufficient to increase the expression of the activity of the target gene or gene expression product. In some instances, the expression or the activity of the gene or gene expression product is increased, relative to that in a normal individual; and administering the rAAV to the subject is sufficient to decrease the expression or the activity of the target gene or gene expression product. In a non-limiting example, a subject diagnosed with Alzheimer's disease, which is caused, in some cases, by a gain-of-function of a Presenilin 1 and/or Presenilin 2 (encoded by the gene PSEN1 and PSEN2, respectively) is administered a rAAV disclosed herein encapsidating a therapeutic nucleic acid that is a silencing RNA (siRNA), or other RNAi with a loss-of-function effect on PSEN1 mRNA.

[0091] Also provided are methods of preventing a disease or condition disclosed herein in a subject comprising administering to the subject a therapeutically effective amount of an rAAV vector comprising a nucleic acid sequence encoding a therapeutic gene expression product described herein. The rAAV vector may be encapsidated in the modified capsid protein or rAAV viral particle described herein. In some instances, the therapeutic gene expression product is effective to modulate the activity or expression of a target gene or gene expression product.

[0092] Disclosed herein are methods of treating a disease or condition in a subject by administering a composition comprising a rAAV disclosed herein. An advantage of the rAAVs disclosed herein, is that the rAAV may be used to treat virtually any disease or condition that would benefit from a transgene therapy, including but not limited to spinal muscular atrophy (SMA), amyotrophic lateral sclerosis (ALS), Parkinson's disease, Pompe disease, mucopolysaccharidosis type II, fragile X syndrome, STXBP1 encephalopathy, Krabbe disease, Huntington's disease, Alzheimer's disease, Battens disease, lysosomal storage disorders, glioblastoma multiforme, Rett syndrome, Leber's congenital amaurosis, Late infantile neuronal ceroid lipofuscinosis (LINCL), chronic pain, stroke, spinal cord injury, traumatic brain injury and lysosomal storage disorders.
[0093] In some cases, the disease or condition is localized to a particular in vivo environment in the subject, e.g., the brain, heart, or brain and heart. The compositions of the present disclosure are particularly useful for the treatment of the diseases or conditions described herein because they specifically or more efficiently target the in vivo environment and deliver a therapeutic nucleic acid engineered to modulate the activity or the expression of a target gene expression product involved with the pathogenesis or pathology of the disease or condition.
[0094] Provided herein are methods of treating a disease or a condition, or a symptom of the disease or condition, in a subject, comprising: (a) diagnosing a subject with a disease or a condition affecting a target in vivo environment; and (b) treating the disease or the condition by administering to the subject a therapeutically effective amount of a composition disclosed herein (e.g., rAAV particle, AAV vector, pharmaceutical composition), wherein the composition is engineered with an increased specificity for the target in vivo environment.
[0095] Disclosed herein are methods of treating a disease or a condition, or a symptom of the disease or the condition, afflicting a target in a subject comprising: (a) administering to the subject a composition (e.g., rAAV particle, AAV vector, pharmaceutical composition); and (b) expressing the therapeutic nucleic acid into a target in vivo environment in the subject with an increased transduction enrichment.
[0096] In some embodiments, methods of treating a disease or condition affecting the brain, heart, or brain and heart comprise administering a rAAV particle to a brain, heart, or brain and heart, respectively, in a subject, the rAAV particle comprising an rAAV capsid protein comprising an insertion of about, five, six, or seven amino acids of an amino acid sequence provided in Tables 1-3 at an amino acid position 588-589 in a parental AAV capsid protein. In some embodiments, methods of treating a disease or condition affecting the brain, heart, or brain and heart comprise administering a rAAV particle to a brain, heart, or brain and heart in a subject, respectively, the rAAV particle comprising an rAAV capsid protein comprising an insertion of about, five, six, or seven amino acids of an amino acid sequence as well as one or more substitution at amino acid found at amino acid positions 587-590 [AQAQ] such as provided in Tables 1-3. In some embodiments, the parental AAV capsid protein is AAV9 capsid protein (for e.g., provided in SEQ ID NO: 1.
[0097] Also provided are methods of modulating a target gene expression product, the methods comprising administering to a subject in need thereof a composition (e.g., rAAV particle, AAV vector, pharmaceutical composition) disclosed herein. For example, methods provided herein comprise administering to a subject a rAAV with a rAAV capsid protein encapsidating a viral vector comprising a heterologous nucleic acid that modulates the expression or the activity of the target gene expression product.
[0098] The term “normal individual” refers to an individual that is not afflicted with the disease or the condition characterized by the variation in expression or activity of the gene or gene expression product thereof.
[0099] In some embodiments, the disease or condition of the brain selected from Absence of the Septum Pellucidum, Acid Lipase Disease, Acid Maltase Deficiency, Acquired Epileptiform Aphasia, Acute Disseminated Encephalomyelitis, Attention Deficit-Hyperactivity Disorder

(ADHD), Adie's Pupil, Adie's Syndrome, Adrenoleukodystrophy, Agenesis of the Corpus Callosum, Agnosia, Aicardi Syndrome, Aicardi-Goutieres Syndrome Disorder, AIDS-Neurological Complications, Alexander Disease, Alpers' Disease, Alternating Hemiplegia, Alzheimer's Disease, Amyotrophic Lateral Sclerosis (ALS), Anencephaly, Aneurysm, Angelman Syndrome, Angiomatosis, Anoxia, Antiphospholipid Syndrome, Aphasia, Apraxia, Arachnoid Cysts, Arachnoiditis, Arnold-Chiari Malformation, Arteriovenous Malformation, Asperger Syndrome, Ataxia, Ataxia Telangiectasia, Ataxias and Cerebellar or Spinocerebellar Degeneration, Atrial Fibrillation and Stroke, Attention Deficit-Hyperactivity Disorder, Autism Spectrum Disorder, Autonomic Dysfunction, Back Pain, Barth Syndrome, Batten Disease, Becker's Myotonia, Behcet's Disease, Bell's Palsy, Benign Essential Blepharospasm, Benign Focal Amyotrophy, Benign Intracranial Hypertension, Bernhardt-Roth Syndrome, Binswanger's Disease, Blepharospasm, Bloch-Sulzberger Syndrome, Brachial Plexus Birth Injuries, Brachial Plexus Injuries, Bradbury-Eggleston Syndrome, Brain and Spinal Tumors, Brain Aneurysm, Brain Injury, Brown-Sequard Syndrome, Bulbospinal Muscular Atrophy, Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL), Canavan Disease, Carpal Tunnel Syndrome, Causalgia, Cavernomas, Cavernous Angioma, Cavernous Malformation, Central Cervical Cord Syndrome, Central Cord Syndrome, Central Pain Syndrome, Central Pontine Myelinolysis, Cephalic Disorders, Ceramidase Deficiency, Cerebellar Degeneration, Cerebellar Hypoplasia, Cerebral Aneurysms, Cerebral Arteriosclerosis, Cerebral Atrophy, Cerebral Beriberi, Cerebral Cavemous Malformation, Cerebral Gigantism, Cerebral Hypoxia, Cerebral Palsy, Cerebro-Oculo-Facio-Skeletal Syndrome (COFS), Charcot-Marie-Tooth Disease, Charcot-Marie-Tooth syndrome, classical rhizomelic chondrodysplasia punctata (RCDP), Chiari Malformation, Cholesterol Ester Storage Disease, Chorea, Choreoacanthocytosis, Chronic Inflammatory Demyelinating Polyneuropathy (CIDP), Chronic Orthostatic Intolerance, Chronic Pain, Cockayne Syndrome, Cockayne Syndrome Type II, Coffin Lowry Syndrome, Colpocephaly, Coma, Complex Regional Pain Syndrome, Congenital Facial Diplegia, Congenital Myasthenia, Congenital Myopathy, Congenital Vascular Cavernous Malformations, Corticobasal Degeneration, Cranial Arteritis, Craniosynostosis, Cree encephalitis, Creutzfeldt-Jakob Disease, Cumulative Trauma Disorders, Cushing's Syndrome, Cytomegalic Inclusion Body Disease, Cytomegalovirus Infection, Dancing Eyes-Dancing Feet Syndrome, Dandy-Walker Syndrome, Dawson Disease, Deafness, De Morsier's Syndrome, Dejerine-Klumpke Palsy, Dementia, Dementia-Multi-Infarct, Dementia-Semantic, Dementia-Subcortical, Dementia With Lewy Bodies, Dentate Cerebellar Ataxia, Dentatorubral Atrophy, Dermatomyositis, Developmental Dyspraxia, Devic's Syndrome, Diabetic Neuropathy, Diffuse Sclerosis, Dravet Syndrome, Duchenne muscular dystrophy, Dysautonomia, Dysgraphia, Dyslexia, Dysphagia, Dyspraxia, Dyssynergia Cerebellaris Myoclonica, Dyssynergia Cerebellaris Progressiva, Dystonias, Early Infantile Epileptic Encephalopathy, Empty Sella Syndrome, Encephalitis, Encephalitis Lethargica, Encephaloceles, Encephalopathy, Encephalopathy (familial infantile), Encephalotrigeminal Angiomatosis, Epilepsy, Epileptic Hemiplegia, Erb's Palsy, Erb-Duchenne and Dejerine-Klumpke Palsies, Essential Tremor, Extrapontine Myelinolysis, Fabry Disease, Fahr's Syndrome, Fainting, Familial Dysautonomia, Familial Hemangioma, Familial Idiopathic Basal Ganglia Calcification, Familial Periodic Paralyses, Familial Spastic Paralysis, Farber's Disease, Febrile Seizures, Fibromuscular Dysplasia, Fisher Syndrome, Floppy Infant Syndrome, Foot Drop, Fragile X syndrome, Friedreich's Ataxia, Frontotemporal Dementia (FTD), Gaucher Disease, Generalized Gangliosidoses, Gerstmann's Syndrome, Gerstmann-Straussler-Scheinker Disease, Giant Axonal Neuropathy, Giant Cell Arteritis, Giant Cell Inclusion Disease, glioblastoma, Globoid Cell Leukodystrophy, Glossopharyngeal Neuralgia, Glycogen Storage Disease, Guillain-Barre Syndrome, Hallervorden-Spatz Disease, Head Injury, Headache, Hemicrania Continua, Hemifacial Spasm, Hemiplegia Alterans, Hereditary Neuropathies, Hereditary Spastic Paraplegia, Heredopathia Atactica Polyneuritiformis, Herpes Zoster, Herpes Zoster Oticus, Hirayama Syndrome, Holmes-Adie

syndrome, Holoprosencephaly, HTLV-1 Associated Myelopathy, Hughes Syndrome, Huntington's Disease, Hydranencephaly, Hydrocephalus, Hydrocephalus-Normal Pressure, Hydromyelia, Hypercortisolism, Hypersomnia, Hypertonia, Hypotonia, Hypoxia, Immune-Mediated Encephalomyelitis, Inclusion Body Myositis, Incontinentia Pigmenti, Infantile Hypotonia, Infantile Neuroaxonal Dystrophy, Infantile Phytanic Acid Storage Disease, Infantile Refsum Disease (IRD), Infantile Spasms, Inflammatory Myopathies, Iniencephaly, Intestinal Lipodystrophy, Intracranial Cysts, Intracranial Hypertension, Isaacs' Syndrome, Joubert Syndrome, Kearns-Sayre Syndrome, Kennedy's Disease, Kinsbourne syndrome, Kleine-Levin Syndrome, Klippel-Feil Syndrome, Klippel-Trenaunay Syndrome (KTS), Kliiver-Bucy Syndrome, Korsakoff s Amnesic Syndrome, Krabbe Disease, Kugelberg-Welander Disease, Kuru, Lambert-Eaton Myasthenic Syndrome, Landau-Kleffner Syndrome, Lateral Femoral Cutaneous Nerve Entrapment, Lateral Medullary Syndrome, Learning Disabilities, Leigh's Disease, Lennox-Gastaut Syndrome, Lesch-Nyhan Syndrome, Leukodystrophy, Levine-Critchley Syndrome, Lewy Body Dementia, Lipid Storage Diseases, Lipoid Proteinosis, Lissencephaly, Locked-In Syndrome, Lou Gehrig's Disease, Lupus-Neurological Sequelae, Lyme Disease-Neurological Complications, Machado-Joseph Disease, Macrencephaly, Maple syrup urine disease, Megalencephaly, Melkersson-Rosenthal Syndrome, Meningitis, Meningitis and Encephalitis, Menkes Disease, Menkes syndrome, Meralgia Paresthetica, Metachromatic Leukodystrophy, Microcephaly, Migraine, Miller Fisher Syndrome, Mini Stroke, Mitochondrial Myopathy, Moebius Syndrome, Monomelic Amyotrophy, Motor Neuron Diseases, Moyamoya Disease, Mucolipidoses, Mucopolysaccharidosis, Mucopolysaccharidosis II, Multi-Infarct Dementia, Multifocal Motor Neuropathy, Multiple Sclerosis, Multiple System Atrophy, Multiple System Atrophy with Orthostatic Hypotension, Muscular Dystrophy, Myasthenia-Congenital, Myasthenia Gravis, Myelinoclastic Diffuse Sclerosis, Myoclonic Encephalopathy of Infants, Myoclonus, Myopathy, Myopathy-Congenital, Myopathy-Thyrotoxic, Myotonia, Myotonia Congenita, Myotonic dystrophy, Narcolepsy, Neuroacanthocytosis, Neurodegeneration with Brain Iron Accumulation, Neurofibromatosis, Neuroleptic Malignant Syndrome, Neurological Complications of AIDS, Neurological Complications of Lyme Disease, Neurological Consequences of Cytomegalovirus Infection, Neurological Manifestations of Pompe Disease, Neurological Sequelae Of Lupus, Neuromyelitis Optica, Neuromyotonia, Neuronal Ceroid Lipofuscinosis, Neuronal Migration Disorders, Neuropathy-Hereditary, Neurosarcoidosis, Neurosyphilis, Neurotoxicity, Nevus Cavernosus, Niemann-Pick Disease, O'Sullivan-McLeod Syndrome, Occipital Neuralgia, Ohtahara Syndrome, Olivopontocerebellar Atrophy, Opsoclonus Myoclonus, Orthostatic Hypotension, Overuse Syndrome, Pain-Chronic, Pantothenate Kinase-Associated Neurodegeneration, Paraneoplastic Syndromes, Paresthesia, Parkinson's Disease, Paroxysmal Choreoathetosis, Paroxysmal Hemicrania, Parry-Romberg, Pelizaeus-Merzbacher Disease, Pena Shokeir II Syndrome, Perineural Cysts, Periodic Paralyses, Peripheral Neuropathy, Periventricular Leukomalacia, Persistent Vegetative State, Pervasive Developmental Disorders, Phenylketonuria, Phytanic Acid Storage Disease, Pick's Disease, Pinched Nerve, Piriformis Syndrome, Pituitary Tumors, Polymyositis, Pompe Disease, Porencephaly, Post-Polio Syndrome, Postherpetic Neuralgia, Postinfectious Encephalomyelitis, Postural Hypotension, Postural Orthostatic Tachycardia Syndrome, Postural Tachycardia Syndrome, Prader-Willi syndrome, Primary Dentatum Atrophy, Primary Lateral Sclerosis, Primary Progressive Aphasia, Prion Diseases, Progressive Hemifacial Atrophy, Progressive Locomotor Ataxia, Progressive Multifocal Leukoencephalopathy, Progressive Sclerosing Poliodystrophy, Progressive Supranuclear Palsy, Prosopagnosia, Pseudo-Torch syndrome, Pseudotoxoplasmosis syndrome, Pseudotumor Cerebri, Psychogenic Movement, Ramsay Hunt Syndrome I, Ramsay Hunt Syndrome II, Rasmussen's Encephalitis, Reflex Sympathetic Dystrophy Syndrome, Refsum Disease, Refsum Disease-Infantile, Repetitive Motion Disorders, Repetitive Stress Injuries, Restless Legs Syndrome, Retrovirus-Associated Myelopathy, Rett Syndrome, Reye's Syndrome, Rheumatic Encephalitis, Riley-Day Syndrome, Sacral Nerve

Root Cysts, Saint Vitus Dance, Salivary Gland Disease, Sandhoff Disease, Schilder's Disease, Schizencephaly, Seitelberger Disease, Seizure Disorder, Semantic Dementia, Septo-Optic Dysplasia, Severe Myoclonic Epilepsy of Infancy (SMEI), Shaken Baby Syndrome, Shingles, Shy-Drager Syndrome, Sjogren's Syndrome. Sleep Apnea, Sleeping Sickness, Sotos Syndrome, Spasticity, Spina Bifida, Spinal Cord Infarction, Spinal Cord Injury, Spinal Cord Tumors, Spinal Muscular Atrophy, Spinocerebellar ataxia, Spinocerebellar Atrophy, Spinocerebellar Degeneration, Steele-Richardson-Olszewski Syndrome, Stiff-Person Syndrome, Striatonigral Degeneration, Stroke, Sturge-Weber Syndrome, STXBP1 encephalopathy. Subacute Sclerosing Panencephalitis, Subcortical Arteriosclerotic Encephalopathy, Short-lasting, Unilateral, Neuralgiform (SUNCT) Headache, Swallowing Disorders, Sydenham Chorea, Syncope, Syphilitic Spinal Sclerosis, Syringohydromyelia, Syringomyelia, Systemic Lupus Erythematosus, Tabes Dorsalis, Tangier disease, Tardive Dyskinesia, Tarlov Cysts, Tay-Sachs Disease, Temporal Arteritis, Tethered Spinal Cord Syndrome, Thomsen's Myotonia, Thoracic Outlet Syndrome, Thyrotoxic Myopathy, Tic Douloureux, Todd's Paralysis, Tourette Syndrome, Transient Ischemic Attack, Transmissible Spongiform Encephalopathies, Transverse Myelitis, Traumatic Brain Injury, Tremor, Trigeminal Neuralgia, Tropical Spastic Paraparesis, Troyer Syndrome, Tuberous Sclerosis, Vascular Erectile Tumor, Vasculitis Syndromes of the Central Nervous Systems, Von Economo's Disease, Von Hippel-Lindau Disease (VHL), Von Hippel-Lindau syndrome, Von Recklinghausen's Disease, Wallenberg's Syndrome, Werdnig-Hoffman Disease, Wernicke-Korsakoff Syndrome, West Syndrome, Whiplash, Whipple's Disease, Williams Syndrome, Wilson Disease, Wolman's Disease, X-Linked Spinal and Bulbar Muscular Atrophy and Zellweger syndrome.

[0100] In some embodiments, the pharmaceutical formulation comprises a therapeutic nucleic acid encoding a therapeutic gene expression product. In some instances, the therapeutic gene expression product is effective to modulate an activity or an expression of a target gene or gene expression product selected from ATP1A2, CACNAIA, SETD5, SHANK3, NF2, DNMT1, TCF4, RAI1, PEX1, ARSA, EIF2B5, EIF2B1, EIF2B2, NPC1, ADAR, MFSD8, STXBP1, PRICKLE2, PRRT2, IDUA, STX1B, Sarcoglycan Alpha (SGCA), glutamic acid decarboxylase 65 (GAD65), glutamic acid decarboxylase 67 (GAD67), CLN2, Nerve Growth Factor (NGF), glial cell derived neurotrophic factor (GDNF), Survival Of Motor Neuron 1, STXBP1, Telomeric (SMN1), Factor X (FIX), Retinoid Isomerohydrolase (RPE65), sarco/endoplasmic reticulum Ca2+-ATPase (SERCA2a), Glucocerebrosidase (GCase), galactocerebrosidase (GALC), CDKL5, Frataxin (FXN), Huntingtin (HTT), methyl-CpG binding protein 2 (MECP2), a peroxisomal biogenesis factor (PEX), progranulin (GRN), an antitubulin agent, copper-zinc superoxide dismutase (SOD1), iduronate 2 sulfatase (hIDS), Glucosylceramidase Beta (GBA), fragile X mental retardation 1 (FMR1), NPC Intracellular Cholesterol Transporter 1 (NPC1), SCN1A, C9orf72, NPS3 and a NLRP3 inflammasome. In some embodiments, the peroxisomal biogenesis factor (PEX) is selected from PEX1, PEX2, PEX3, PEX4, PEX5, PEX6, PEX7, PEX10, PEX11B, PEX12, PEX13, PEX14, PEX16, PEX19, and PEX26.

[0101] In some aspects, other examples of genes involved in neurologic or brain diseases or disorders include MAPT, IDUA, SNCA, ATXN2, Ube3a, GNS, HGSNAT, NAGLU, SGSH, CLN1, CLN3, CLN4, CLN5, CLN6, CLN7, CLN8, CTSD, ABCD1, HEXA, HEXB, ASM, ASPA, GLB1, AADC, MFN2, GNAO1, SYNGAP1, GRIN2A, GRIN2B, KCNQ2, EPM2A, NHLRC1, SLC6A1, SLC13A5, SURF1, GBE1, ATXN1, ATXN3, and ATXN7.

[0102] In some embodiments, the disease or condition of the heart includes any disease of the cardiopulmonary system, including, but not limited to heart failure, ischemia, arrhythmia, myocardial infarction, congestive heart failure, transplant rejection, abnormal heart contractility, non-ischaemic cardiomyopathy, mitral valve regurgitation, refractory myocardial ischaemia, non-ischaemic heart failure, aortic stenosis or regurgitation, abnormal Ca2+ metabolism, dysregulation of a heart-specific protein or a protein effective in modulating cardiac activity or physiology.

[0103] In some embodiments, genes involved in heart disease include those the express proteins

involved in the regulation of calcium cycling in cardiomyocytes, such as a sarcoplasmic endoplasmic reticulum Ca2+ ATPase pump.

[0104] In some embodiments, cells of the heart or heart tissue comprise tissue or cells of any portion of the cardiopulmonary system. In certain aspects, cells of the heart or heart tissue comprises cardiac muscle cells/tissue, cells/tissue of the cardiac vasculature, and cells/tissue present in a cardiac valve. Cells of the heart may include cardiomyocytes, epithelial cells, endothelial cells, fibroblasts, cells of the conducting tissue, cardiac pacemaking cells, and neurons.

[0105] In some instances, the therapeutic gene expression product comprises gene editing components. In some instances, the gene editing components are selected from an artificial site-specific RNA endonuclease (ASRE), a zinc finger endonuclease (ZFN), a transcription factor like effector nuclease (TALEN), a clustered regularly interspaced short palindromic repeats (CRISPR)/Cas enzyme, and a CRISPR)/Cas guide RNA.

[0106] In some instances, the expression of a gene or expression or activity of a gene expression product is inhibited by the administration of the composition to the subject. In some instances, the expression of a gene or the expression or the activity of a gene expression product is enhanced by the administration of the composition to the subject.

Formulations, Dosages, and Routes of Administration

[0107] Disclosed herein are methods comprising delivering a rAAV particle encapsidating a heterologous nucleic acid to the brain, heart, or brain and heart in a subject, the rAAV particle comprising (i) an increased transduction of the heterologous nucleic acid in the brain, heart, or brain and heart, wherein the rAAV particle has an rAAV capsid protein comprising an insertion of five, six, or seven amino acids of an amino acid sequence provided in Tables 1-3, at an amino acid position 588-589 in a parental AAV capsid protein as well as one or more substitution at amino acid found at amino acid positions 587-590 [AQAQ] such as provided in Tables 1-3. In various embodiments, the rAAV capsid protein may comprise one or more substitutions at amino acid positions 452-458 alone or in combination with the modifications above.

[0108] In general, methods disclosed herein comprise administering a therapeutic rAAV composition by systemic administration. In some instances, methods comprise administering a therapeutic rAAV composition by intravenous (“i.v.”) administration. One may administer therapeutic rAAV compositions by additional routes, such as subcutaneous injection, intramuscular injection, intradermal injection, transdermal injection, percutaneous administration, intranasal administration, intralymphatic injection, rectal administration intragastric administration, intraocular administration, intracerebroventricular administration, intrathecally, intracisternal, or any other suitable parenteral administration. Routes, dosage, time points, and duration of administrating therapeutics may be adjusted. In some embodiments, administration of therapeutics is prior to, or after, onset of either, or both, acute and chronic symptoms of the disease or condition. Other routes of delivery to the brain, heart, or brain and heart include, but are not limited to intracranial administration, lateral cerebroventricular administration, and endovascular administration.

[0109] An effective dose and dosage of pharmaceutical compositions to prevent or treat the disease or condition disclosed herein is defined by an observed beneficial response related to the disease or condition, or symptom of the disease or condition. Beneficial response comprises preventing, alleviating, arresting, or curing the disease or condition, or symptom of the disease or condition. In some embodiments, the beneficial response may be measured by detecting a measurable improvement in the presence, level, or activity, of biomarkers, transcriptomic risk profile, or intestinal microbiome in the subject. An “improvement,” as used herein refers to shift in the presence, level, or activity towards a presence, level, or activity, observed in normal individuals (e.g individuals who do not suffer from the disease or condition). In instances wherein the therapeutic rAAV composition is not therapeutically effective or is not providing a sufficient alleviation of the disease or condition, or symptom of the disease or condition, then the dosage

amount and/or route of administration may be changed, or an additional agent may be administered to the subject, along with the therapeutic rAAV composition. In some embodiments, as a patient is started on a regimen of a therapeutic rAAV composition, the patient is also weaned off (e.g., step-wise decrease in dose) a second treatment regimen.

[0110] In some cases, a dose of the pharmaceutical composition may comprise a concentration of infectious particles of at least or about 10.sup.7, 10.sup.8, 10.sup.9, 10.sup.10, 10.sup.11, 10.sup.12, 10.sup.13, 10.sup.14, 10.sup.15, 10.sup.16, or 10.sup.17. In some cases, the concentration of infectious particles is 2×10.sup.7, 2×10.sup.8, 2×10.sup.9, 2×10.sup.10, 2×10.sup.11, 2×10.sup.12, 2×10.sup.13, 2×10.sup.14, 2×10.sup.15, 2×10.sup.16, or 2×10.sup.17. In some cases, the concentration of the infectious particles is 3×10.sup.7, 3×10.sup.8, 3×10.sup.9, 3×10.sup.10, 3×10.sup.11, 3×10.sup.12, 3×10.sup.13, 3×10.sup.14, 3×10.sup.15, 3×10.sup.16, or 3×10.sup.17. In some cases, the concentration of the infectious particles is 4×10.sup.7, 4×10.sup.8, 4×10.sup.9, 4×10.sup.10, 4×10.sup.11, 4×10.sup.12, 4×10.sup.13, 4×10.sup.14, 4×10.sup.15, 4×10.sup.16, or 4×10.sup.17. In some cases, the concentration of the infectious particles is 5×10.sup.7, 5×10.sup.8, 5×10.sup.9, 5×10.sup.10, 5×10.sup.11, 5×10.sup.12, 5×10.sup.13, 5×10.sup.14, 5×10.sup.15, 5×10.sup.16, or 5×10.sup.17. In some cases, the concentration of the infectious particles is 6×10.sup.7, 6×10.sup.8, 6×10.sup.9, 6×10.sup.10, 6×10.sup.11, 6×10.sup.12, 6×10.sup.13, 6×10.sup.14, 6×10.sup.15, 6×10.sup.16, or 6×10.sup.17. In some cases, the concentration of the infectious particles is 7×10.sup.7, 7×10.sup.8, 7×10.sup.9, 7×10.sup.10, 7×10.sup.11, 7×10.sup.12, 7×10.sup.13, 7×10.sup.14, 7×10.sup.15, 7×10.sup.16, or 7×10.sup.17. In some cases, the concentration of the infectious particles is 8×10.sup.7, 8×10.sup.8, 8×10.sup.9, 8×10.sup.10, 8×10.sup.11, 8×10.sup.12, 8×10.sup.13, 8×10.sup.14, 8×10.sup.15, 8×10.sup.16, or 8×10.sup.17. In some cases, the concentration of the infectious particles is 9×10.sup.7, 9×10.sup.8, 9×10.sup.9, 9×10.sup.10, 9×10.sup.11, 9×10.sup.12, 9×10.sup.13, 9×10.sup.14, 9×10.sup.15, 9×10.sup.16, or 9×10.sup.17.

[0111] Disclosed herein, in some embodiments are formulations of pharmaceutically-acceptable excipients and carrier solutions suitable for delivery of the rAAV compositions described herein, as well as suitable dosing and treatment regimens for using the particular compositions described herein in a variety of treatment regimens. In some embodiments, the amount of therapeutic gene expression product in each therapeutically-useful composition may be prepared is such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

[0112] In some embodiments, the pharmaceutical forms of the rAAV-based viral compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.

[0113] In some cases, for administration of an injectable aqueous solution, the solution may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. Some variation in dosage will necessarily occur

depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by FDA Office of Biologics standards.

[0114] Disclosed herein are sterile injectable solutions comprising the rAAV compositions disclosed herein, which are prepared by incorporating the rAAV compositions disclosed herein in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. Injectable solutions may be advantageous for systemic administration, for example by intravenous or intrathecal administration.

[0115] Suitable dose and dosage administrated to a subject is determined by factors including, but not limited to, the particular therapeutic rAAV composition, disease condition and its severity, the identity (e.g., weight, sex, age) of the subject in need of treatment, and can be determined according to the particular circumstances surrounding the case, including, e.g., the specific agent being administered, the route of administration, the condition being treated, and the subject or host being treated.

[0116] The amount of rAAV compositions and time of administration of such compositions will be within the purview of the skilled artisan having benefit of the present teachings. It is likely, however, that the administration of therapeutically-effective amounts of the disclosed compositions may be achieved by a single administration, for example, a single injection of sufficient numbers of infectious particles to provide therapeutic benefit to the patient undergoing such treatment. This is made possible, at least in part, by the fact that certain target cells (e.g., neurons) do not divide, obviating the need for multiple or chronic dosing.

[0117] In certain embodiments, the data obtained from cell culture assays and animal studies are used in formulating the therapeutically effective daily dosage range and/or the therapeutically effective unit dosage amount for use in mammals, including humans. In certain embodiments, the dosage range and/or the unit dosage amount varies within this range depending upon the dosage form employed and the route of administration utilized.

Combination Therapies

[0118] A therapeutic rAAV may be used alone or in combination with an additional therapeutic agent (together, “therapeutic agents”). In some cases, a therapeutic rAAV as used herein is administered alone. The therapeutic agent may be administered together or sequentially in a combination therapy. The combination therapy may be administered within the same day, or may be administered one or more days, weeks, months, or years apart.

[0119] The additional therapeutic agent can comprise a small molecule. The additional therapeutic agent can comprise an antibody, or antigen-binding fragment. The additional therapeutic agent can include lipid nanoparticle-based therapies, anti-sense oligonucleotide therapies, as well as other viral therapies.

[0120] The additional therapeutic agent can comprise a cell-based therapy. Exemplary cell-based therapies include without limitation immune effector cell therapy, chimeric antigen receptor T-cell (CAR-T) therapy, natural killer cell therapy and chimeric antigen receptor natural killer (NK) cell therapy. Either NK cells, or CAR-NK cells, or a combination of both NK cells and CAR-NK cells can be used in combination with the methods disclosed herein. In some embodiments, the NK cells and CAR-NK cells are derived from human induced pluripotent stem cells (iPSC), umbilical cord blood, or a cell line. The NK cells and CAR-NK cells can comprise a cytokine receptor and a

suicide gene. The cell-based therapy can comprise a stem cell therapy. The stem cell therapy may be embryonic or somatic stem cells. The stem cells may be isolated from a donor (allogeneic) or isolated from the subject (autologous). The stem cells may be expanded adipose-derived stem cells (eASCs), hematopoietic stem cells (HSCs), mesenchymal stem (stromal) cells (MSCs), or induced pluripotent stem cells (iPSCs) derived from the cells of the subject.

Kits

[0121] Disclosed herein are kits comprising compositions disclosed herein. Also disclosed herein are kits for the treatment or prevention of a disease or conditions of the brain, heart, or brain and heart. In some instances, the disease or condition is cancer, a pathogen infection, pulmonary disease or condition, neurological disease, muscular disease, or an immune disorder, such as those described herein.

[0122] In one embodiment, a kit can include a therapeutic or prophylactic composition containing an effective amount of a composition of a rAAV particle encapsidating a recombinant AAV vector encoding a therapeutic nucleic acid (e.g., therapeutic nucleic acid) and a recombinant AAV (rAAV) capsid protein of the present disclosure. In another embodiment, a kit can include a therapeutic or prophylactic composition containing an effective amount of cells modified by the TAAV described herein ("modified cell"), in unit dosage form that express therapeutic nucleic acid. In some embodiments, a kit comprises a sterile container which can contain a therapeutic composition; such containers can be boxes, ampules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

[0123] In some instances, the kit further comprises a cell. In some instances, the cell is mammalian. In some instances, the cell is immortalized. In some instances, the immortalized cell is an embryonic stem cell. In some instances, the embryonic stem cell is a human embryonic stem cell. In some instances, the human embryonic stem cell is a human embryonic kidney 293 (HEK-293) cell. In some instances, the kit further comprises an AAV vector comprising a heterologous nucleic acid encoding a therapeutic gene expression product. In some instances, the AAV vector is an episome.

[0124] In some cases, rAAV are provided together with instructions for administering the rAAV to a subject having or at risk of developing the disease or condition (e.g., disease of the brain, heart, or brain and heart). Instructions can generally include information about the use of the composition for the treatment or prevention of the disease or condition.

[0125] In some cases, the instructions include at least one of the following: description of the therapeutic rAAV composition; dosage schedule and administration for treatment or prevention of the disease or condition disclosed herein; precautions; warnings; indications; counter-indications; overdosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions can be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container. In some cases, instructions provide procedures for administering the rAAV to the subject alone. In some instances, the instructions provide that the rAA Vis formulated for systemic delivery.

Definitions

[0126] The terminology used herein is for the purpose of describing particular cases only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising."

[0127] The term "about" or "approximately" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how

the value is measured or determined, e.g., the limitations of the measurement system. For example, “about” can mean within 1 or more than 1 standard deviation, per the practice in the given value. Where particular values are described in the application and claims, unless otherwise stated the term “about” should be assumed to mean an acceptable error range for the particular value.

[0128] As used herein “consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the stated purpose. Thus, a composition consisting essentially of the elements as defined herein would not exclude other materials or steps that do not materially affect the basic and novel characteristic(s) of the claimed disclosure, such as compositions for treating skin disorders like acne, eczema, psoriasis, and rosacea.

[0129] The terms “homologous,” “homology,” or “percent homology” are used herein to generally mean an amino acid sequence or a nucleic acid sequence having the same, or similar sequence to a reference sequence. Percent homology of sequences can be determined using the most recent version of BLAST, as of the filing date of this application.

[0130] The terms “increased,” or “increase” are used herein to generally mean an increase by a statically significant amount. In some embodiments, the terms “increased,” or “increase,” mean an increase of at least 10% as compared to a reference level, for example an increase of at least about 10%, at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% increase or any increase between 10-100% as compared to a reference level, standard, or control. Other examples of “increase” include an increase of at least 2-fold, at least 5-fold, at least 10-fold, at least 20-fold, at least 50-fold, at least 100-fold, at least 1000-fold or more as compared to a reference level.

[0131] The terms, “decreased” or “decrease” are used herein generally to mean a decrease by a statistically significant amount. In some embodiments, “decreased” or “decrease” means a reduction by at least 10% as compared to a reference level, for example a decrease by at least about 20%, or at least about 30%, or at least about 40%, or at least about 50%, or at least about 60%, or at least about 70%, or at least about 80%, or at least about 90% or up to and including a 100% decrease (e.g., absent level or non-detectable level as compared to a reference level), or any decrease between 10-100% as compared to a reference level. In the context of a marker or symptom, by these terms is meant a statistically significant decrease in such level. The decrease can be, for example, at least 10%, at least 20%, at least 30%, at least 40% or more, and is preferably down to a level accepted as within the range of normal for an individual without a given disease.

[0132] The terms “subject” is any organism. In some instances, the organism is a mammal. Non-limiting examples of mammal include, any member of the mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. In certain aspects, the mammal is a human. The term “animal” as used herein comprises human beings and non-human animals. In one embodiment, a “non-human animal” is a mammal, for example a rodent such as rat or a mouse. In one embodiment, a “non-human primate” is a mammal, for example a monkey. In some instances, the subject is a patient, which as used herein, may refer to a subject diagnosed with a particular disease or disorder.

[0133] The term “gene,” as used herein, refers to a segment of nucleic acid that encodes an individual protein or RNA (also referred to as a “coding sequence” or “coding region”), optionally together with associated regulatory region such as promoter, operator, terminator and the like, which may be located upstream or downstream of the coding sequence.

[0134] The term “adeno-associated virus,” or “AAV” as used herein refers to the adeno-associated virus or derivatives thereof. Non-limited examples of AAV's include AAV type 1 (AAV1), AAV

type 2 (AAV2), AAV type 3 (AAV3), AAV type 4 (AAV4), AAV type 5 (AAV5), AAV type 6 (AAV6), AAV type 7 (AAV7), AAV type 8 (AAV8), AAV type 9 (AAV9), AAV type 10 (AAV10), AAV type 11 (AAV11), AAV type 12 (AAV12), avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. In some instances, the AAV is described as a "Primate AAV," which refers to AAV that infect primates. Likewise an AAV may infect bovine animals (e.g., "bovine AAV", and the like). In some instances, the AAV is wildtype, or naturally occurring. In some instances, the AAV is recombinant.

[0135] The term "AAV capsid" as used herein refers to a capsid protein or peptide of an adeno-associated virus. In some instances, the AAV capsid protein is configured to encapsidate genetic information (e.g., a transgene, therapeutic nucleic acid, viral genome). In some instances, the AAV capsid of the instant disclosure is a modified AAV capsid, relative to a corresponding parental AAV capsid protein.

[0136] The term "tropism" as used herein refers to a quality or characteristic of the AAV capsid that may include specificity for, and/or an increase or a decrease in enrichment of, expressing the encapsidated genetic information into an in vivo environment, relative to a second in vivo environment. An in vivo environment, in some instances, is a cell-type. An in vivo environment, in some instances, is an organ or organ system.

[0137] The term "AAV vector" as used herein refers to nucleic acid polymer encoding genetic information related to the virus. The AAV vector may be a recombinant AAV vector (rAAV), which refers to an AAV vector generated using recombinatorial genetics methods. In some instances, the rAAV vector comprises at least one heterologous polynucleotide (e.g. a polynucleotide other than a wild-type or naturally occurring AAV genome such as a transgene).

[0138] The term "AAV particle" as used herein refers to an AAV virus, virion, AAV capsid protein or component thereof. In some cases, the AAV particle is modified relative to a parental AAV particle.

[0139] The term "gene product" of "gene expression product" refers to an expression product of a polynucleotide sequence such as, for e.g., a polypeptide, peptide, protein or RNA, including interfering RNA (e.g., siRNA, miRNA, shRNA) and messenger RNA (mRNA).

[0140] The term "heterologous" as used herein refers to a genetic element (e.g., coding region) or gene expression product (e.g., RNA, protein) that is derived from a genotypically distinct entity from that of the rest of the entity to which it is being compared.

[0141] The term "endogenous" as used herein refers to a genetic element (e.g., coding region) or gene expression product (e.g., RNA, protein) that is naturally occurring in or associated with an organism or a particular cell within the organism.

[0142] The terms "treat," "treating," and "treatment" as used herein refers to alleviating or abrogating a disorder, disease, or condition; or one or more of the symptoms associated with the disorder, disease, or condition; or alleviating or eradicating a cause of the disorder, disease, or condition itself. Desirable effects of treatment can include, but are not limited to, preventing occurrence or recurrence of disease, alleviation of symptoms, diminishing any direct or indirect pathological consequences of the disease, preventing metastasis, decreasing the rate of disease progression, amelioration or palliation of the disease state and remission or improved prognosis.

[0143] The term "therapeutically effective amount" refers to the amount of a compound or therapy that, when administered, is sufficient to prevent development of, or alleviate to some extent, one or more of the symptoms of a disorder, disease, or condition of the disease; or the amount of a compound that is sufficient to elicit biological or medical response of a cell, tissue, system, animal, or human that is being sought by a researcher, veterinarian, medical doctor, or clinician.

[0144] The term "pharmaceutically acceptable carrier," "pharmaceutically acceptable excipient," "physiologically acceptable carrier," or "physiologically acceptable excipient" refers to a pharmaceutically acceptable material, composition, or vehicle, such as a liquid or solid filler, diluent, excipient, solvent, or encapsulating material. A component can be "pharmaceutically

acceptable" in the sense of being compatible with the other ingredients of a pharmaceutical formulation. It can also be suitable for use in contact with the tissue or organ of humans and animals without excessive toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. See, Remington: The Science and Practice of Pharmacy, 21st Edition; Lippincott Williams & Wilkins: Philadelphia, PA, 2005; Handbook of Pharmaceutical Excipients, 5th Edition; Rowe et al., Eds., The Pharmaceutical Press and the American Pharmaceutical Association: 2005; and Handbook of Pharmaceutical Additives, 3rd Edition; Ash and Ash Eds., Gower Publishing Company: 2007; Pharmaceutical Preformulation and Formulation, Gibson Ed., CRC Press LLC: Boca Raton, FL, 2004).

[0145] The term "pharmaceutical composition" refers to a mixture of a compound disclosed herein with other chemical components, such as diluents or carriers. The pharmaceutical composition can facilitate administration of the compound to an organism. Multiple techniques of administering a compound exist in the art including, but not limited to, systemic administration.

[0146] Non-limiting examples of "sample" include any material from which nucleic acids and/or proteins can be obtained. As non-limiting examples, this includes whole blood, peripheral blood, plasma, serum, saliva, mucus, urine, semen, lymph, fecal extract, cheek swab, cells or other bodily fluid or tissue, including but not limited to tissue obtained through surgical biopsy or surgical resection. Alternatively, a sample can be obtained through primary patient derived cell lines, or archived patient samples in the form of preserved samples, or fresh frozen samples.

[0147] The term "in vivo" is used to describe an event that takes place in a subject's body.

[0148] The term "in vitro" is used to describe an event that takes places contained in a container for holding laboratory reagent such that it is separated from the biological source from which the material is obtained. In vitro assays can encompass cell-based assays in which living or dead cells are employed. In vitro assays can also encompass a cell-free assay in which no intact cells are employed.

[0149] The term "brain" means a tissue selected from brain, thalamus, cortex, putamen, lateral ventricles, medulla, the pons, the amygdala, the motor cortex, caudate, hypothalamus, striatum, ventral midbrain, neocortex, basal ganglia, hippocampus, cerebrum, cerebellum, brain stem, and spinal cord. The brain includes a variety of cortical and subcortical areas, including the frontal, temporal, occipital and parietal lobes.

[0150] The term "systemic delivery" is defined as a route of administration of medication or other substance into a circulatory system so that the entire body is affected. Administration can take place via enteral administration (absorption of the drug through the gastrointestinal tract) or parenteral administration (generally injection, infusion, or implantation). "Circulatory system" includes both blood or cerebrospinal fluid circulatory systems. Examples of systemic administration for the brain include intraarterial, intravenous or intrathecal injection. Other examples include administration to the cerebrospinal fluid at any location, in the spine (i.e. but not limited to lumbar) or brain (i.e. but not limited to cisterna magna). The terms "systemic administration" and "systemic delivery" are used interchangeably.

[0151] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described.

[0152] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

[0153] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent

application was specifically and individually indicated to be incorporated by reference.
EXAMPLES
Example 1
Method of Identifying the Modified Capsid Proteins in Cynomolgus Macaques
[0154] Following IV injection, unmodified AAV9 does not have sufficient tissue enrichment to treat many human diseases that would otherwise be amenable to treatment via delivery of an AAV cargo of 4.7 kbp DNA. Directed evolution of AAV9 was performed as a means of increasing viral tissue enrichment to levels that enable disease treatment.
[0155] Adeno-Associated Virus serotype 9 (AAV9) was modified by inserting a stretch of 7 amino acids (7mer) following residue #588 in its capsid coding sequence. The 7mer was randomly generated via degenerate DNA basepairs, thus sampling all 207 possible 7mer amino acid sequences. Three batches of this library with billions of variants were prepared separately.
[0156] One batch was IV injected into three African Green Monkeys, then three weeks later the animals were sacrificed and tissue samples were screened with PCR to uncover the best 7mer sequences that caused improved enrichment relative to AAV9 in the brain and heart. The other batch was IV injected into two Cynomologus Macaque Non-Human Primates, then two weeks later the animals were sacrificed and tissue samples were screened with PCR to uncover the best 7mer sequences that caused improved enrichment relative to AAV9 in the brain and heart. The third batch was delivered identically to the second batch and data were analyzed identically, but in a separate study using different Cynomolgus Macaques.
[0157] Following these three PCR screenings of tissues, the top 41,000 variants identified in the NHP brains and hearts were brought forward into a ‘round 2’ screening. This smaller library was IV injected into three Cynomologus Macaque Non-Human Primates, then two weeks later the animals were sacrificed and tissue samples were screened with PCR to uncover the best 7mer sequences that caused further improved enrichment relative to AAV9 in the brain and heart.

Claims

1. A mutant Adeno-Associated Virus (AAV) capsid protein comprising at least 95% sequence identity to the wild-type AAV capsid protein, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-1496.
2. The mutant AAV capsid protein of claim 1, wherein the mutant AAV capsid protein comprises 7-mer amino acid insertion sequence located between residues 588 and 589 in relation to the wild-type AAV capsid protein.
3. The mutant AAV capsid protein of claim 1, wherein the mutant AAV capsid protein comprises at least 95% sequence identity to the wild-type Adeno-Associated Virus serotype 9 (AAV9) capsid of SEQ ID NO: 1.
4. The mutant AAV capsid protein of claim 1, wherein the amino acids immediately preceding the 7-mer amino acid insertion sequence are -AQ-.
5. The mutant AAV capsid protein of claim 1, wherein the amino acids immediately following the 7-mer amino acid insertion sequence are -AQ-.
6. The mutant AAV capsid protein of claim 1, wherein 60 copies of the AAV capsid protein are assembled into the AAV capsid.
7. The mutant AAV capsid protein of claim 1, wherein the AAV capsid protein is present in VP1, VP2, and VP3 of the mutant AAV capsid.
8. The mutant AAV capsid protein of claim 1, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-898.
9. The mutant AAV capsid protein of claim 8, further characterized by an increased transduction enrichment relative to the wild-type AAV9 when measured in brain tissue in a subject when delivered to the subject systemically.

10. The mutant AAV capsid protein of claim 9, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-594.
11. The mutant AAV capsid protein of claim 1, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 595-1496.
12. The mutant AAV capsid protein of claim 11, further characterized by an increased transduction enrichment relative to the wild-type AAV9 when measured in heart tissue in a subject when delivered to the subject systemically.
13. The mutant AAV capsid protein of claim 12, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 899-1496.
14. The mutant AAV capsid protein of claim 1, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 595-898.
15. The mutant AAV capsid protein of claim 14, further characterized by an increased transduction enrichment relative to the wild-type AAV9 when measured in brain tissue and heart tissue in a subject when delivered to the subject systemically.
16. An AAV capsid comprising a mutant AAV capsid protein comprising at least 95% sequence identity to the wild-type AAV capsid protein, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-1496.
17. The AAV capsid of claim 16, wherein the AAV capsid is chimeric.
18. The AAV capsid of claim 17, wherein the capsid is isolated and purified.
19. The AAV capsid of claim 16, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-898.
20. The AAV capsid of claim 19, further characterized by an increased transduction enrichment relative to the wild-type AAV9 when measured in brain tissue in a subject when delivered to the subject systemically.
21. The AAV capsid of claim 20, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-594.
22. The AAV capsid of claim 16, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 595-1496.
23. The AAV capsid of claim 22, further characterized by an increased transduction enrichment relative to the wild-type AAV9 when measured in heart tissue in a subject when delivered to the subject systemically.
24. The AAV capsid of claim 23, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 899-1496.
25. The AAV capsid of claim 16, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 595-898.
26. The AAV capsid of claim 25, further characterized by an increased transduction enrichment relative to the wild-type AAV9 when measured in brain tissue and heart tissue in a subject when delivered to the subject systemically.
27. A composition comprising an AAV capsid, wherein the AAV capsid comprises an AAV capsid protein comprising at least 95% sequence identity to the wild-type AAV capsid protein, wherein the mutant AAV capsid protein comprises a sequence selected from the group consisting of SEQ ID NO: 2-1496.
28. The composition of claim 27, formulated for systematic administration to a subject.
29. The composition of claim 28, wherein systematic administration of the composition results in expression of a gene product delivered by the AAV capsid.
30. The composition of claim 29, wherein administration of the composition results in increased transduction enrichment relative to the wild-type AAV9 when measured in heart and/or brain.
31. A method of treating a disease or condition in a subject comprising administering a therapeutically effective amount of a composition of claim 27.

32. The composition of claim 27, for use in treating a disease or condition in a subject, the use comprising administering a therapeutically effective amount of a composition of claim 27.